The Hidden Powers of Glomalin: Insights into Soil Health and Functionality

Abstract:

Agricultural practices such as the application of inorganic fertilizers and pesticides have profound effects on soil, altering its physical and chemical properties and consequently impacting soil biota composition and diversity. Since plant health is intricately linked to soil health, managing soil in a manner that conserves and enhances soil biota can significantly improve crop yield and quality. The rhizosphere, the zone directly influenced by plant roots, harbours high populations of active microorganisms and plays a vital role in plant-microbe interactions. One of the most crucial symbiotic relationships in the rhizosphere is between plants and arbuscular mycorrhizal fungi (AMF). AMF form mutualistic associations with the roots of various plant species, including major crops and contribute to defence against soil-borne pathogens, nutrient cycling and soil aggregation. Glomalin, a glycoprotein secreted by AMF, plays a crucial role in soil aggregation, stability and carbon sequestration. It enhances soil structure, binds with soil particles, stabilizes aggregates and promotes water infiltration and retention. It also plays a role in sequestering toxic metals, reducing their availability to plants and mitigating their harmful effects on soil biota. Managing glomalin in soil involves practices such as minimizing tillage to preserve the hyphal network, maintaining living roots through cover crops to sustain fungal colonization and optimizing nutrient inputs to support fungal activity without overloading the soil. AMF and glomalin play pivotal roles in agricultural sustainability and soil ecosystem functioning, highlighting the importance of conserving and enhancing these beneficial microbial associations for improved soil health and crop productivity.

Key words: Glomalin, Arbuscular Mycorrhizal Fungi, Soil Aggregation, Nutrient Cycling

Introduction

"Agricultural practices, including the use of inorganic fertilizers and pesticides can significantly alter the physical and chemical properties of the soil. These changes can affect the abundance and diversity of soil organisms. Because plant health is closely connected to soil health, managing the soil in ways that support and enhance its biological community can lead to better crop yields and quality" (Napoli *et al*., 2008). The rhizosphere, often referred to as the "heart of the soil" is the zone directly influenced by plant roots and characterized by high populations of active microorganisms. In this critical

region, plant roots shape microbial communities by releasing photosynthates, while these microorganisms, in turn regulate plant growth and development (Dhillion and Gardsiord, 2004). The association between plants and arbuscular mycorrhizal fungi (AMF) represents one of the most crucial symbiotic relationships on earth, connecting the root system with the soil.

AMF are widespread, root-symbiotic fungi that typically form mutualistic relationships with the roots of higher plants, including major crop and pasture species. The term "mycorrhizae" originates from the Greek words "mycos" meaning fungus and "rhiza" meaning root. AMF play a vital role in defending against soil-borne pathogens and are involved in essential processes such as nutrient cycling and soil aggregation.

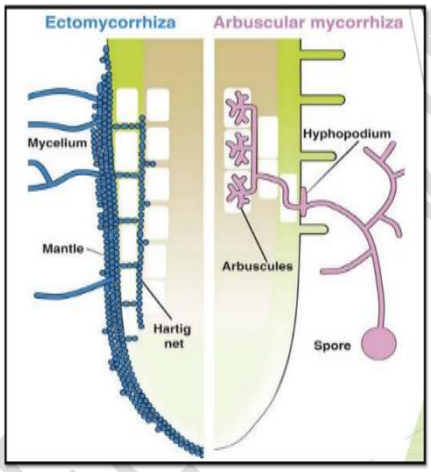

Fig 1. Root-symbiotic fungi

AMF exist in two different phases:

1. Ectomycorrhiza (in soil)
2. Endomycorrhiza (inside root cells)

- **Ectomycorrhiza:** Ectomycorrhiza refers to a type of symbiotic relationship between the roots of certain plants and fungi. In this association, the fungal hyphae form a dense sheath or mantle around the surface of the plant roots and extend into the soil, enhancing nutrient and water absorption. The fungal hyphae also penetrate between root cells, forming a network called the Hartig net, which facilitates nutrient exchange between the fungus and the plant.
- **Endomycorrhiza:** Endomycorrhiza, also known as arbuscular mycorrhiza (AM), is a type of symbiotic relationship between fungi and the roots of most terrestrial plants. In this association, the fungal hyphae penetrate the root cell walls and form structures called arbuscules and vesicles within the root cells. These structures facilitate the exchange of nutrients between the plant and the fungus.

1. Arbuscules: Fungi forms shrub shaped structure inside the root cortical cell by branching several very thin hyphae. This is where the exchange of nutrients and carbon happens between the host plant and the fungus.
2. Vesicles: Vesicles are structures formed by the fungal hyphae within the root cells of the host plant. Vesicles serve as storage organs, accumulating lipids and other nutrients that can be used by both the fungus and the plant during periods of nutrient scarcity.
3. Hyphae: Root-like structure that grow outside the root, in long distances to explore the soil for nutrient uptake.

"AMF form hyphal networks that can contain over 100 m of hyphae per cubic centimeter of soil and are important for nutrient uptake and soil aggregation. Among the different species most dominated species is *Glomus* and the reason behind it may be the *Glomus* species can propagate from both spores and broken pieces of hyphae" (Bird *et al*., 2002). Significant advances in research on mycorrhizal physiology and ecology have greatly enhanced our understanding of the various roles of AMF in ecosystems. One key discovery is the role of glomalin, a glue-like protein produced by AMF, which significantly contributes to soil structure improvement by promoting soil aggregation.

What is Glomalin?

Glomalin is a fungal protein, or a class or group of proteins, that is operationally quantified from soil as glomalin-related soil protein. Initially believed to be exuded by living fungi, it is now understood that glomalin is released into the soil environment during hyphal turnover and after the death of AMF. However, the exact relationship between glomalin and these soil protein fractions has not been clearly established yet.

How does glomalin work?

"Glomalin contributes to soil tilth, giving it a smooth texture that experienced farmers and gardeners can identify by the way soil granules flow through their fingers. In AMF-plant symbioses, AMF transfer nutrients from the soil to the plant via the extraradical mycelium, while the plant supplies AMF with carbon in the form of photosynthates, ranging from 5-85% of the plant's carbon, depending on the species" (Brundrett, 2004). "Besides nutrient uptake, the extraradical mycelium is involved in spore formation and root colonization. The primary benefit for plants in this symbiotic relationship is enhanced nutrient uptake from the soil" (Cappellazzo *et al*., 2008). As hyphae cease nutrient transport, their protective glomalin is released into the soil, binding mineral particles and organic matter to form stable clods. This soil structure is resilient against wind and water erosion while remaining porous enough to allow air, water, and roots to penetrate.

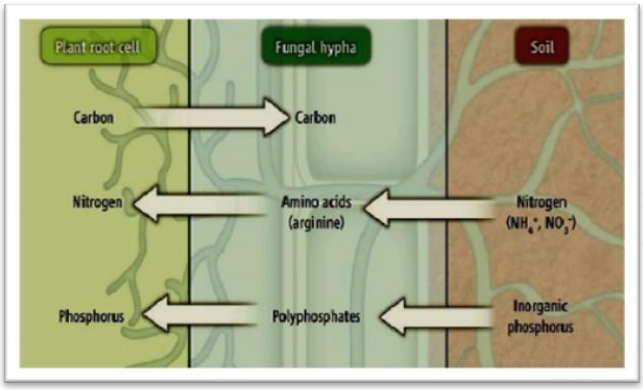

Fig 2. Principle of glomalin

History:

Glomalin was discovered in 1996 by Sara F. Wright, a soil scientist at the United States Department of Agriculture's Agricultural Research Service. Initially mistaken for an unidentified component of soil organic matter, glomalin is actually a "soil super glue" that permeates organic matter, binding it to silt, sand and clay particles. Its concentration in soil is strongly positively correlated with the water-stability of soil aggregates. In 1998, Wright and her colleague employed various citric acid buffers to extract glomalin from the soil, categorizing it based on its solubility.

i. Easily extractable
ii. Total extractable

Origin of glomalin:

"The investigation into glomalin commenced with the identification of a monoclonal antibody (Mab32B11) that exhibits immunological reactivity on the surfaces of *Glomus intraradices* spores. This protein originates from the intraradical hyphae within plant roots and the surface of extraradical hyphae in the rhizosphere, and it can be released from the mycelial surface into the soil. Glomalin is thought to be the sole protein directly secreted by AMF into the soil" (Rillig *et al*, 1999).

Current terminologies for glomalin and their definitions

- **Glomalin-related soil protein (GRSP):** The total soil glomalin fraction, which may include other soil proteins. It is extracted repeatedly using a 50 M sodium citrate solution (pH 8) and autoclaving at 121 °C for 60 minutes until the extract is straw-colored.
- **Easily extractable glomalin-related soil protein (EE-GRSP):** The fraction of soil glomalin extracted once using a 20 M sodium citrate solution (pH 7) and autoclaving at 121 °C for 30 minutes.
- **Bradford-reactive soil protein (BRSP):** Glomalin-related soil protein quantified using the Bradford assay, which measures all proteins in the glomalin extract.

- **Easily extractable Bradford-reactive soil protein (EE-BRSP):** Easily extractable glomalin-related soil protein quantified using the Bradford assay, measuring all proteins in the glomalin extract.
- **Immunoreactive soil protein (IRSP):** Glomalin-related soil protein quantified using an indirect enzyme-linked immunosorbent assay (ELISA) with the monoclonal antibody MAb32B11, which is specific for glomalin but may cross-react with other soil proteins.
- **Easily extractable immunoreactive soil protein (EE-IRSP):** Easily extractable glomalin-related soil protein quantified using an indirect ELISA with the monoclonal antibody MAb32B11.

Evidence that GRSP is (at least partly) of AMF Origin:

"There are several notable challenges in glomalin research; however, it is important to clarify that these challenges do not pertain to the operational definition of glomalin-related soil protein (GRSP) itself, but rather to the association between GRSP and AMF. Accumulating circumstantial evidence from decomposition studies suggests that GRSP originates from AMF. When AMF growth is inhibited, such as by incubating soil without host plants, a decline in GRSP concentrations along with AMF hyphae is observed" (Steinberg and Rillig, 2003). "This experimental design leverages the fact that AMF are obligate biotrophs, unlike most other soil fungi. Interestingly, the hyphal lengths of other saprobic fungi and bacterial biomass increased during the study" (Steinberg and Rillig, 2003). A similar decrease in GRSP concentrations was noted after more than 400 days of soil incubation from various land use types (forest, afforested area, and agricultural land) (Rillig *et al*., 2003). In long-term grassland plots treated with fungicide to eliminate AMF, GRSP concentrations (both BRSP and IRSP) significantly decreased (Rillig *et al*., 2003).

"While these decomposition studies do not definitively rule out the presence of other cross-reactive materials in soil, they establish a strong link between AMF and GRSP, even amidst the activities of non-biotrophic soil biota that could produce potentially cross-reactive material. The use of a monoclonal antibody (MAb32B11) for detecting IRSP (and EE-IRSP) further supports the AMF origin of these GRSP fractions, as MAb32B11 is less likely to cross-react with many other epitopes compared to a polyclonal antibody, unless it targets a relatively common structure or there are issues with non-specific binding. This antibody has shown strong reactivity with all tested AMF species" (Wright and Upadhyaya, 1996; Rillig, 2004), and minimal cross-reactivity with other soil fungal isolates. The antibody's reaction is not likely an artifact of harsh extraction conditions, as MAb32B11 can also visualize material on AMF spores and hyphae *in-situ* and in soil (Wright and Upadhyaya, 1996).

"Additionally, IRSP production was observed under sterile, soil-free *in-vitro* conditions using transformed root organ cultures, where IRSP was measured in the hypha-only compartment of split plate cultures" (Steinberg and Rillig, 2003). In subsequent experiments, IRSP was also found to accumulate in

the liquid culture medium over time (Driver *et al*., 2005). These findings reinforce that AMF produce IRSP and exclude hypha-associated bacteria as likely sources of GRSP, although they do not entirely eliminate the possibility of cross-reactive sources in soil.

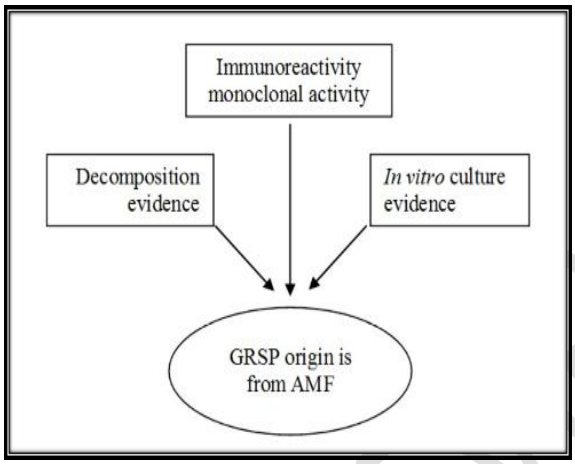

Fig 3. Immunoreactivity activity

Structure and composition:

"Glomalin is a stable and persistent glycoprotein released by hyphae and spores of AMF in the Glomales taxon. It is resistant to trypsin and chemical hydrolysis. Glomalin contains iron, has N-linked oligosaccharides and is insoluble and possibly hydrophobic in its native state. It resists heat degradation due to its glue-like nature, binding to the surface of spores" (Wright and Upadhyaya, 1998). Despite being an N-linked glycoprotein, its biochemical structure is not fully defined. The elemental composition of glomalin includes:

Nitrogen (N): 3-5%

Carbon (C): 36-59%

Hydrogen (H): 4-6%

Oxygen (O): 33-49%

Phosphorus (P): 0.03-0.1%

Iron (Fe): 0.8-8.8%

Identification:

The identification of glomalin is based on its solubility characteristics, categorized into:

1) Easily extractable glomalin

2) Total glomalin

3) A “scum” component (likely a sloughed part of glomalin)

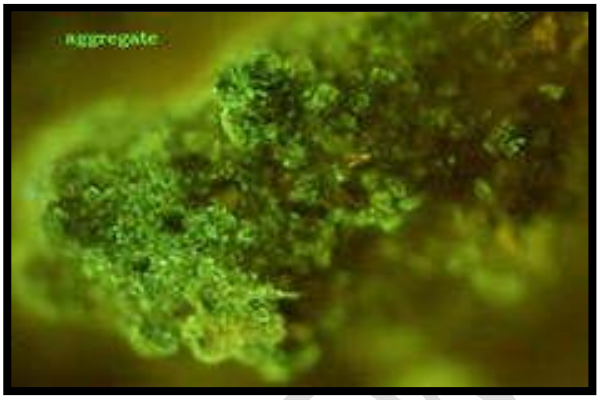

Fig 4 a & 4b Identification of glomalin

“Soil organic matter, metals (like iron), clay minerals, and other substances may bind to glomalin, causing conformational changes. It appears dark red-brown and soil loses its brown color after extraction due to the removal of organic matter. Unusually high extraction quantities leave the soil a mineral grey color. Laboratory procedures reveal glomalin on soil aggregates as green material” (Wright and Upadhyaya, 1996).

The current extraction protocol is based on Wright’s method. For clarity, Koide and People (2013) divided GRSP into fraction 1 (easily extractable) and fraction 2 (older, more difficult to extract). Wu (2014) proposed naming fraction 1 as EE-GRSP (easily extractable glomalin-related soil protein) and fraction 2 as DE-GRSP (difficultly extractable glomalin-related soil pr otein).

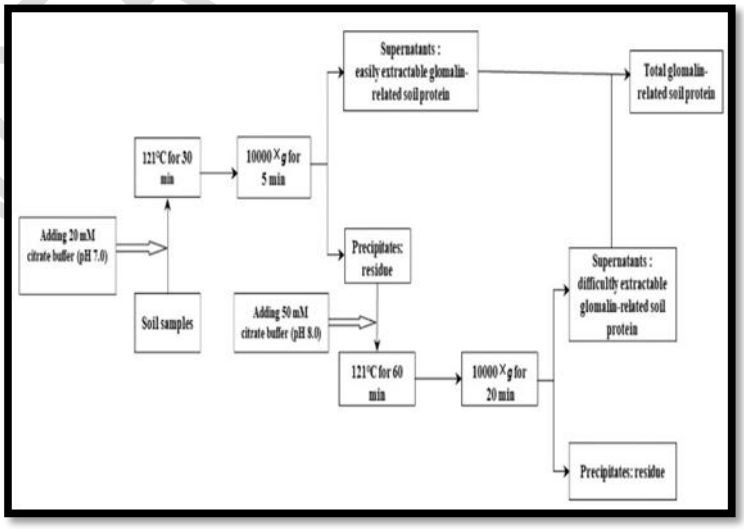

Fig 5:The extraction protocol of different glomalin-related soil protein fractions in soils

For analysis:

- One gram of soil is mixed with 8 mL of 20 mmol/L citrate (pH 7.0) and incubated at 121°C and 0.11 MPa for 30 minutes, then centrifuged at 10,000 x g for 5 minutes. The supernatant is EE-GRSP.
- The residue is mixed with 10 mL of 50 mmol/L citrate (pH 8.0), incubated at 121°C for 60 minutes, and centrifuged at 10,000 x g for 20 minutes. The supernatant is DE-GRSP.
- The sum of EE-GRSP and DE-GRSP is T-GRSP (total glomalin-related soil protein) (Fokom *et al*., 2012).

Note: GRSP is extracted from field soil, root, mesh, or pot culture media.

Quantification:

Extracted glomalin can be quantified using two different methods:

1. Bradford protein analysis method
2. ELISA method

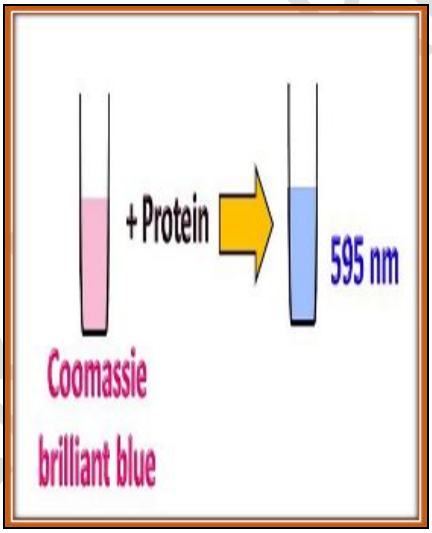

Fig 6. Quantification with two different methods

1) Bradford protein analysis method: Glomalin is defined by the method used to quantify it (Wright *et al*., 1996; Rillig, 2004). The Bradford protein analysis is a widely used method for protein quantification (Bradford, 1976). "This assay is based on the principle that Coomassie Brilliant Blue G-250 dye binds to proteins, causing a color change from red to blue" (Bradford, 1976; Steinberg and Rillig, 2003). "The degree of this color change, measured by a spectrophotometer at a wavelength of 590 nm (A590), correlates with protein concentration in a glomalin extract. The assay uses a standard curve prepared with bovine serum albumin (BSA) ranging from 1.25 to 5 μg in phosphate-buffered saline (PBS). The regression equation from plotting optical density against BSA values is used to calculate protein concentration in glomalin extracts, termed Bradford-reactive soil protein (BRSP) for total glomalin (TG)

and easily extractable Bradford-reactive soil protein (EE-BRSP) for the easily extractable glomalin (EEG) fraction" (Rillig, 2004)

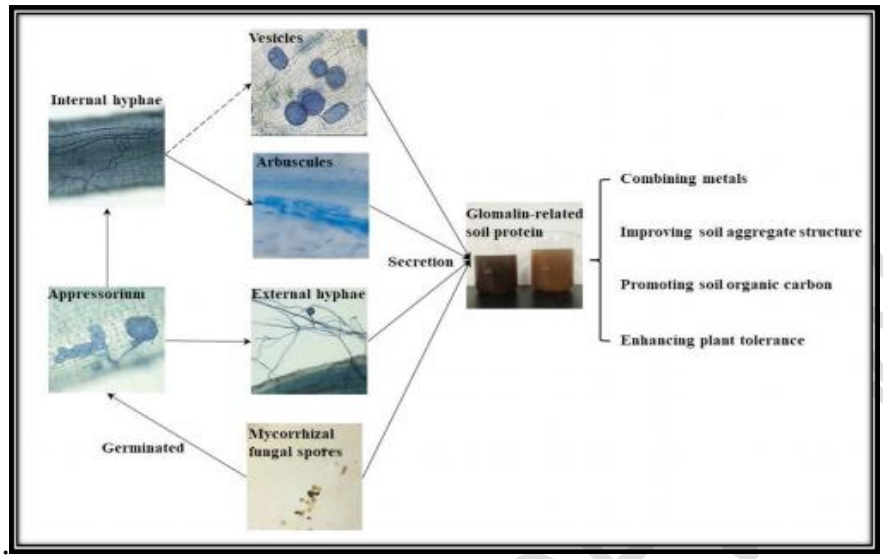

Fig 7. Bradford protein analysis method

2) ELISA (**Enzyme-linked immunosorbent assay**): The ELISA technique, also known as enzyme immunoassay, is used to detect and quantify specific compounds like antigens, antibodies and hormones. It measures the amount of a compound by the conversion of a substrate to a colored product by an enzyme-linked antibody. This method is quick and sensitive, allowing for the quantification of many samples in parallel. Glomalin content in samples is determined by indirect ELISA using the monoclonal antibody Mab32B11, produced against spores of Glomus intraradices. Total glomalin (TG) quantified using ELISA is referred to as immunoreactive soil protein (IRSP) and the easily extractable fraction is named easily extractable immunoreactive soil protein (EE-IRSP) (Wright and Upadhyaya, 1998; Rillig, 2004). The extraction procedure does not remove other soil proteins. EE-BRSP and T-IRSP have been positively correlated with soil available nitrogen (N) and phosphorus (P) (Wu *et al*., 2014). Monoclonal antibodies have shown high specificity in differentiating between fungal isolates.

Potential functions of glomalin:

"AMF spores germinate, extend and eventually form an appressorium to contact root epidermal cells of host plants. These colonized hyphae continue into the cortical cells of roots, where arbuscules are formed. Internal root hyphae extend outward to form external hyphae on roots, absorbing nutrients and water from the soil" (Wright and Upadhyaya, 1999). Arbuscules, vesicles, external hyphae and spores secrete GRSP, which functions as follows:

1) GRSP Functioning in Soil Organic Carbon:

The carbon stored in GRSP is highly recalcitrant, lasting for 12–22 years (Zou *et al*., 2016), and plays a significant role in soil structure development (Chi *et al*., 2018). "AMF consume 4–20% of photosynthates from host plants, depositing carbon into the soil through extraradical hyphae, contributing to soil organic carbon (SOC) pools" (Zhu and Miller, 2003). Positive correlations between GRSP and SOC indicate that GRSP is a crucial source of carbon for SOC build-up (Wright and Upadhyaya, 1998; Rillig *et al*., 2003). GRSP's carbon content is 2–24 times higher than that of soil humus, accounting for up to 27% of SOC (Rillig *et al*., 1999). In highly saline soils, purified T-GRSP has a high carbon content (43.41%) (Zhang *et al*., 2017).

2) GRSP functioning on soil aggregate stability

"Soil aggregation is influenced by various factors, including soil organic matter, moisture content, microbial activity, root development, tillage and fertilization. Aggregates are clusters of soil particles held together by stronger forces than those between adjacent aggregates. Glomalin contributes to soil aggregation by binding soil particles, providing a hydrophobic coating and sloughing off hyphae onto organic matter" (Harner *et al*., 2004). This stabilizes aggregates, protecting them from disruption by rainfall and erosion, thus preserving soil nutrients and structure.

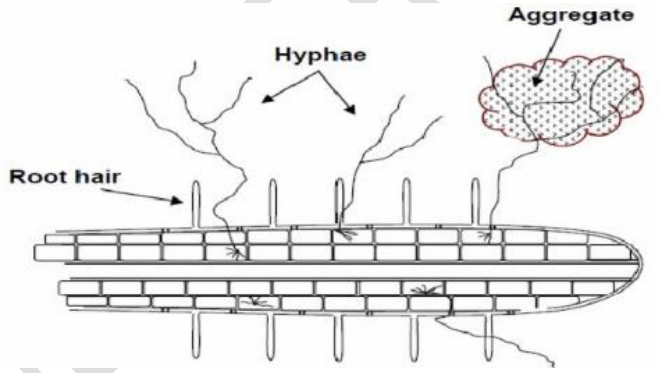

Fig. 8: Hyphae of AMF form a frame for soil particles to collect into aggregates which are coated with glomalin

"Hyphae provide a framework for soil particles to adhere to, while glomalin acts as a glue, binding and protecting these particles. This can be likened to constructing a house: hyphae serve as the framework (akin to wall studs), soil particles act as the insulation filling the spaces, microbial glues such as glomalin and polysaccharides from fungi and bacteria function as the wallboard that stabilizes everything and glomalin itself forms a protective coating, similar to paint" (Preger *et al*., 2007). The aggregation of soil particles is a crucial role for glomalin and other microbial polysaccharides.

Glomalin is essential for stabilizing soil aggregates. Without stabilization aggregates can disintegrate under rainfall leading to the loss of organic matter and nutrients through erosion. The chemical properties of glomalin make it an ideal stabilizing agent. It increases the contact angle for water penetration, which limits infiltration and slaking, reduces wettability and enhances the internal cohesion of aggregates.

3) GRSP Relationship with Soil Aggregate Water Stability:

"Understanding the relationship between GRSP and soil aggregate water stability requires recognizing the varying degrees of water stability in different soils. Once the GRSP concentration in a soil reaches a certain threshold or "saturation" point, additional GRSP does not further enhance soil aggregate water stability when assessed with conventional disintegration methods" (Preger *et al*., 2007). "The aggregation pattern in soils with high GRSP levels follows a curvilinear trend, where many of the pores within macro-aggregates are partially sealed by GRSP, reducing water penetration into the aggregates. This relationship is significant primarily in hierarchically structured soils, where organic material is the main binding agent. In contrast, soils where carbonates serve as the primary binding agent show no positive correlation between GRSP fractions and aggregate stability" (Harner *et al*., 2004).

4) GRSP functioning on soil toxic elements:

Toxic metal accumulation in soil adversely affects plant growth and crop yield and poses risks to human health through the food chain. AMF mitigate the toxicity of these metals to plants by accumulating, converting and transferring them. GRSP exhibit a buffering capacity for toxic metal release, thereby protecting host plants and possessing a high binding affinity for metals such as copper (Cu), cadmium (Cd) and lead (Pb). GRSP stabilize these metals and reduce their bioavailability, consequently minimizing their impact on other soil microorganisms and plants.

Soil organic matter (SOM), including fulvic acid (FA) and humic acid (HA), plays a crucial role in binding potentially toxic elements thereby alleviating metal toxicity and influencing metal behavior in soils. Humin (HM), an insoluble component of SOM also significantly contributes to this metal-binding process. Despite the established roles of FA, HA and HM, there is limited information on the contribution of metal-bound GRSP within SOM fractions and how metal binding affects glomalin decomposition. Research by Gonzalez-Chavez *et al*. (2004) demonstrates that GRSP can stabilize metals such as Pb, Cd, zinc (Zn), Cu, iron (Fe) and manganese (Mn) thereby contributing to the remediation of polluted soils.

5) Exogenous GRSP application in crops:

Exogenous GRSP plays a crucial role in enhancing soil aggregate stability and soil phosphatase activity. Soil tillage significantly disrupts the mycorrhizal hyphal network, adversely impacting GRSP production and subsequently diminishing its role in aggregate stabilization. However, the application of exogenous easily extractable GRSP (EE-GRSP) has shown beneficial effects on drought tolerance. This is due to glomalin's function as a humic-like substance that stimulates plant growth, acts similarly to Hsp60 as a stress moderator, forms a hydrophobic layer on fungal hyphae to prevent water loss and increases plant iron superoxide dismutase (Fe-SOD) activity due to its iron content (Filho *et al*., 2002).

Advantages of glomalin:

1. Protect hyphae from nutrient loss
2. Glue soil aggregate and stabilizes it
3. Reduces wind and water erosion
4. Increases water infiltration
5. Increases water retention near roots
6. Improves root penetration by reducing compaction

Management of glomalin in soil:

Soil management to increase aggregation must aim at increasing primary plant production, increasing the amount of C input into the soil, decreasing disturbances and decreasing the rate of C loss by processes such as decomposition and erosion. In this regard, improved management practices include tillage methods, residue management, amendments, soil fertility management and nutrient cycling.

1. **Minimum or no-till to reduce disruption of hyphal network**: Use no-till management practices to allow AMF to grow during the cropping season. Tillage disrupts the hyphal network that produces glomalin. Disruption of the hyphal network also decreases the number of spores and hyphae to start the process again on the next crop.
2. **Cover crops to maintain living roots**: Use cover crops to maintain living roots for the fungi to colonize
3. **Reduced inputs, minimum Phosphorus**: Maintain adequate phosphorus level for crops, but does not over-apply P because high levels depress the activity of these fungi.

Conclusion:

AMF play an important role in agroecosystem including the involvement of the extraradical mycelium in providing soil aggregation. GRSP has be shown to be correlated with soil aggregate water stability, although the mechanisms underlying this pattern are not yet understood and it can absorb toxic metals in the soil to decrease the damage of toxic metals on plant. Even though GRSP are beneficial still there is a need to analyse the structure and constituents of glomalin.

Data availability Statement

This review article draws from a wide array of current and seminal sources, ensuring comprehensive coverage and up-to-date insights on the subject matter.

References:

Bird S B, Herrick J E, Wander M M and Wright S F, 2002, Spatial heterogeneity of aggregatestability and soil carbon in semi-arid rangeland. *Environmental Pollut*ion 116, 445-455.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgramquantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry* 72, 248-252.

Brundrett MC (2004) Co-evolution of roots and mycorrhizas of land plants, *New Phytologist* 154, 275-304.

Cappellazzo G, Lanfranco L, Fitz M, Wipf D, Bonfante P (2008) Characterization of anamino acid permease from the endomycorrhizal fungus Glomus mosseae. *Plant Physiologist* 147, 429-437.

Chi GG, Srivastava AK, Wu QS (2018) Exogenous easilyextractable glomalin-related soil protein improves droughttolerance of trifoliate orange. *Archives of Agronomy and Soil Science* 64, 1341-1350.

Dhillion SS, Gardsjord TL (2004) Arbuscular mycorrhizas influence plant diversity, productivity and nutrients in boreal grasslands. *Canadian Journal of Botany* 82(1), 104-114.

Driver JD, Holben WE, Rillig MC (2005) Characterization of glomalin as a hyphalwall component of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry* 37, 101-106.

Filho CC, Lourenco A, Guimaraes MDF, Fonseca ICB (2002) Aggregate stability underdifferent soil management systems in a red Latosol in the state of Parana, Brazil. *Soil Tillage and Research* 65, 45-55.

Fokom R, Adamou S, Teugwa MC, BegoudeBoyogueno AD, Nana WL, Ngonkeu MEL, Tchameni NS, Nwaga D, Tsala Ndzomo G, Amvam ZPH (2012) Glomalin related soil protein, carbon, nitrogen and soil aggregate stability as affected by land usevariation in the humid forest zone of south Cameroon. *Soil Tillage and Research* 120, 69-75.

Gonzalez-Chavez MC, Carrillo-Gonzalez R, Weight SF, Nichols KA (2004) The role of glomalin, a protein produced by arbuscular mycorrhizal fungi, in sequestering potentially toxic elements. *Enviromental Pollution* 130, 317-323.

Harner MJ, Ramsey PW, Rillig MC (2004) Protein accumulation and distribution in flood plain soil and river foam. *Ecology Letters* 7, 829-836.

Koide RT, People MS (2013) Behavior of Bradford-reactivesubstances is consistent with predictions for glomalin. *Applied Soil Ecology* 63, 8-14.

Napoli C, Mello A, Bonfante P (2008) Dissecting the rhizosphere complexity, the truffle-ground study case. *Rendiconti Lincei* 19, 241-259.

Preger AC, Rillig MC, Johns AR, Du-Preez CC, Lobe I, Amelung W (2007) Losses of glomalin-related soil protein under prolonged arable cropping, A chronosequencestudy in sandy soils of the South African Highveld. *Soil Biology and Biochemistry* 93, 454-453.

Rillig MC (2004) Arbuscular mycorrhizae, glomalin and soil quality. *Canadian Journal of Soil Science* 84, 355-363.

Rillig MC, Ramsey PW, Morris S, Paul EA (2003) Glomalin, an Arbuscular-mycorrhizal fungal soil protein, responds to land-use change. *Plant Soil* 253, 293-299.

Rillig MC, Wright SF, Allen MF, Field CB (1999) Rise in carbon dioxide changes soil structure. *Nature* 400, 628.

Steinberg PD, Rillig MC (2003) Differential decomposition of arbuscular mycorrhizal fungal hyphae and glomalin. *Soil Biology and Biochemistry* 35, 191-194.

Wright SF, Upadhyaya A (1996) Extraction of an abundant and unusual protein from soil andcomparison with hyphal protein of arbuscular mycorrhizal fungi. *Soil Science* 161, 575-586.

Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, aglycoprotein produced by hyphae of arbuscular mycorrhizal fungi. *Plant Soil* 198, 97-107.

Wright SF, Upadhyaya A (1999) Quantification of arbuscular mycorrhizal fungi activity by the glomalin concentration on hyphal traps. *Mycorrhiza* 8, 283-285.

Wright SF, Franke-Snyder M, Morton JB, Upadhyaya A (1996) Time-course study and partialcharacterization of a protein on hyphae of arbuscular mycorrhizal fungi during activecolonization of roots. *Plant and Soil* 181, 193-203.

Wu Z, Kim M, Jidong H, Pengbao W, Weidong W, Hailong W (2014) Decomposition and the contribution of glomalin-related soil protein (GRSP) in heavy metal sequestration: Field experiment. *Soil Biology and Biochemistry* 68, 283-290.

Zhang ZH, Wang Q, Wang H, Nie S, Liang ZW (2017) Effects of soil salinity on the content, composition and ionbinding capacity of glomalin-related soil protein (GRSP). *Science of the Total Environment* 581, 657-665.

Zhu YG, Miller RM (2003) Carboncycling by arbuscular mycorrhizal fungi in soilplant systems. *Trends Plant Science* 8, 407-409.

Zou YN, Srivastava AK, Wu QS (2016) Glomalin: a potential soil conditioner for perennial fruits. *International Journal of Agriculture and Biology* 18(2), 293-297.

Nonato ERL, Leite JA, Silva JM, Nóbrega CMB, Silva J do N, Antas RN, Ribeiro IR. Mycorrhizal Fungi and their Importance in the Conservation of the Caatinga. J. Exp. Agric. Int. [Internet]. 2022 Apr. 28 [cited 2024 May 17];44(6):19-24. Available from: https://journaljeai.com/index.php/JEAI/article/view/1944

Waratadar A, Nirmalnath PJ, Matiwade PS, Navi V. Soil Enzyme Activity and Nutrient Uptake as Influenced by Arbuscular Mycorrhizal Fungi in Tobacco Rhizosphere under Parasitic Weed Stress Condition. Int. J. Plant Soil Sci. [Internet]. 2022 Aug. 24 [cited 2024 May 17];34(22):984-95. Available from: https://journalijpss.com/index.php/IJPSS/article/view/2368

Andrade G, Mihara KL, Linderman RG, Bethlenfalvay GJ. Bacteria from rhizosphere and hyphosphere soils of different arbuscular-mycorrhizal fungi. Plant and soil. 1997 May;192:71-9.