**_Review Article_**

# Role of Glomalin in Improving Soil Fertility: A Review


## ABSTRACT

Mycorrhizal fungi are found naturally in undisturbed soils around the world. They form symbiotic relationships with almost all plants ranging from ornamentals, fruits, vegetables, trees and shrubs. Most of the plants have a strong dependency on mycorrhizal fungi for optimal growth. The mycorrhizal symbiosis is a key stone to the productivity and diversity of natural plant ecosystems. The symbiotic relationships are found between fungi and plants and the most prevalent plant symbiosis known and as a result VAM symbiosis is found in more than 80% of vascular plant families. Glomalin-related soil protein component is produced by arbuscular mycorrhiza, and as stable glue the hyphae has an important role in soil aggregate stabilization. The glomalin produced from some crop rotation cropping system could promote aggregate stability.

Glomalin binds to soil, producing a uniform aggregated structure composed of minerals and humus. Increasing organic matter increases cation exchange capacity of soils. Primarily, these aggregates permit the soil to retain water better and facilitate root penetration. In addition, the aggregates reduce soil erosion and compaction while facilitating root hair adhesion, enhancing nutrient and water uptake.



*Keywords:*:- *Mycorrhizal fungi,* arbuscular mycorrhiza *(AM)*, *Symbiosis,* Glomalin-related soil protein, aggregate


## 1. INTRODUCTION

Agricultural practices such as adding inorganic fertilizers and pesticides can change the physical and chemical nature of the soil environment, there by altering the number of organisms and the ratio of different groups of organisms. Since plant health is intimately linked to soil health, managing the soil in ways that conserve and enhance the soil biota can improve crop yields and quality. A diverse soil community will not only help to prevent losses due to soil-borne pests and diseases but also speed up decomposition of organic matter and toxic compounds, and improve nutrient cycling and soil structure. The rhizosphere is the zone under the direct influence of the plant roots and with high populations of active microorganisms. In the rhizosphere plant roots influence microbial communities by depositing photosynthate into the rhizosphere and organisms growing up plant growth and development.[1]

Microorganisms are the most abundant members of the soil biota. The wide range of organisms that inhabit soil play important roles in driving many of the key terrestrial biogeochemical cycles that underwrite primary production, via the provision of mineral nutrients to plants. They include species responsible for nutrient mineralization and cycling, antagonists (biological control agents against plant pests and diseases), species that produce substances capable of modifying plant growth, and species that form mutually beneficial (symbiotic) relationships with plant roots.[2]

The tremendous advances in research on mycorrhizal physiology and ecology over the past 40 years have led to a greater understanding of the multiple roles of vesicular arbuscular mycorrhizal (VAM) in the ecosystem. There are number of situations where manipulation or management of the mycorrhizal symbiosis is necessary to restore plant cover, improve plant health or increase plant productivity.[3] Thus, mycorrhizal technology becomes an important consideration in low input, organic or soil-less agriculture. The desire to exploit VAM (glomalin protein) as a natural biofertilizers for the agricultural biotechnology industries are understandable, but it became clear that more knowledge is needed of the fungi themselves to allow commercial exploitation. The benefit of the symbiosis for nutrient uptake by plants in agro-ecosystems is important as the knowledge is applicable to human endeavors for ecosystem management, restoration and sustainability. In view of this, more complete understanding of how to manage vesicular arbuscular mycorrhiza (Glycoprotein) for optimum plant growth, health and development is needed urgently as high-input plant production practices are challenged by a more sustainable biological production approaches.

## 2. ARBUSCULAR MYCORRHIZAL FUNGI

An arbuscular mycorrhizal fungus is a type of mycorrhiza in which the fungus penetrates the cortical cells of the roots of a vascular plant. Arbuscular mycorrhizas (AMs) are characterized by the formation of unique structures, arbuscules and vesicles by fungi of the phylum Glomeromycota (AM fungi).

The most common and best known of these associations are the Vesicular Arbuscular Mycorrhizae (VAM). Vesicular Arbuscular Mycorrhizas are produced by aseptate mycelial fungi and are so-called because of the two characteristic structures-vesicles and arbuscules- found in roots with type of infection.[4] They are of general occurrence in the *Gramineae, Palmae, Rosaceae and Leguminosae*, which all include many crop plants and in land plants, possesses this type of mycorrhiza. It is believed that the development of the VAM symbiosis played a crucial role in the initial colonization of the land by plants and in evolution of the vascular plants.[5]

### 2.1 Symbiotic association of VAM with plant

The association between plants and AMF is one of the most important symbioses on earth, linking the root and the soil system.[6] Arbuscular mycorrhizal symbiosis is possibly the oldest and the most abundant plant-microbe association on earth. They play a crucial role in agricultural systems by increasing plant tolerance to abiotic and biotic stresses.[7] They increase plant growth, improve salt and drought tolerance, and potentially improve heavy metal tolerance. The symbiosis also plays a role in nutrient cycling in soil, in ecosystem productivity, and plant variety.[8]

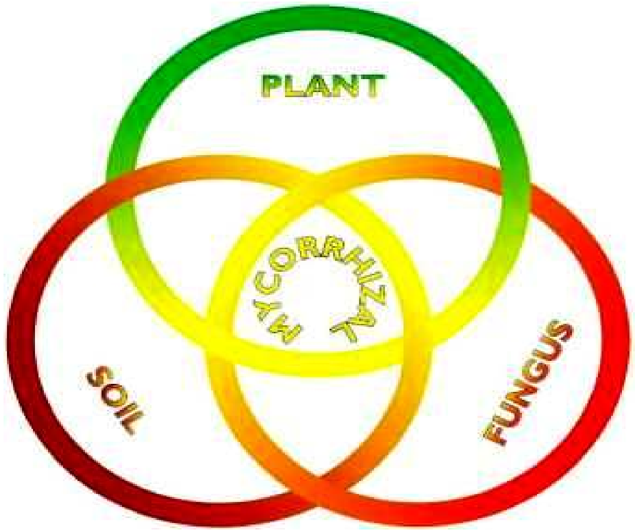


Figure 2.1- Mycorrhizal association, showing the interactions between fungus, plant and soil.[9]

Three main components  are involved in VAM association: 1) the soil, 2) the fungus and 3) the plant (Figure 2.1). The fungal component involves, the fungal structure within the cell of the root and the extraradical mycelium in the soil. The last  may be quite extensive under some conditions, but does not form any vegetative structures. Its primary function is the absorption of resources from the soil. The increased efficiency of mycorrhizal roots versus nonmycorrhizal roots is caused by the active uptake and transport of nutrients by mycorrhizae. Primarily, nutrient and Carbon exchanges between AMF and plant occur in the arbuscules, while the vesicles, where present, are a storage organ. Also, AMF possess intraradical hyphae located within the host and extraradical hyphae found outside the root, in the soil environment. Collectively, the, arbuscules, vesicles, and intraradical hyphae are regarded as the intraradical mycelium, and the collection of extraradical hyphae is known as extraradical mycelium.[10]

In AMF-plant symbioses, AMF translocate nutrients from soil to plant through the extraradical mycelium, and in return, the plant supplies AMF with Carbon in the form of photosynthates; about 5 to 85% of Carbon depending on the plant species. Apart from nutrient uptake, the extraradical mycelium also is involved in spore formation and initiation of root colonization. Spores, hyphae, and colonized root and organic matter are propagules of AMF.[11] The significance of mycorrhizal symbiosis in the nutrition and well-being of the individual plant is well established.

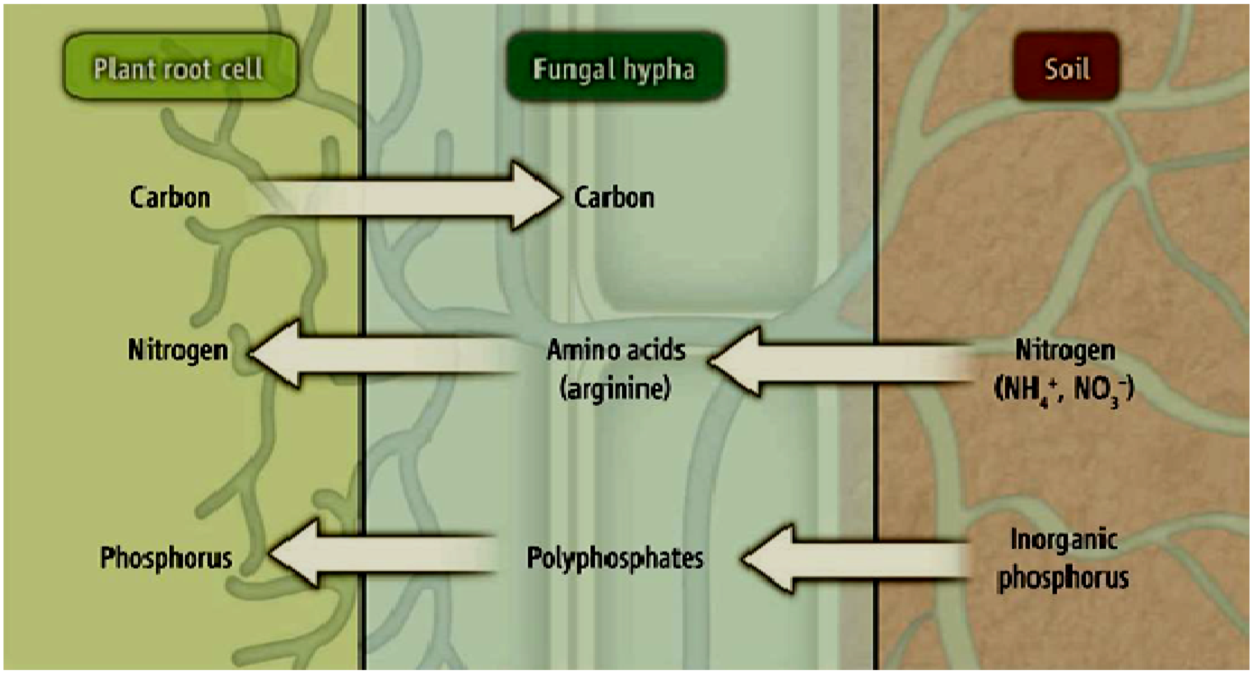


Figure 2.2- The benefits of plant-fungal symbiosis are stabilized by the constant of mutual nutrient supply.[12]

The major benefit for plants when being mycorrhizal is an increase in plant nutrient uptake from the soil (Figure 2.2). Inorganic and organic nutrients are absorbed by extraradical hyphae from the soil through specific transporters of phosphate, ammonium, nitrogen, amino acids, zinc, and copper. All of these are subsequently moved to the plant roots.[13]

The most pronounced benefit for plant nutrient uptake through VAM has been described for nutrient which is limited mobility in the soil such as phosphorus. Not only the uptake of (Phosphorus) P is enhanced by VAM colonization of plant roots, the uptake of other macro and micronutrients have also been enhanced. Enhancements in the acquisition of K, Ca and Mg are often observed in VAM colonized plants grown on acidic soils than neutral or alkaline soils.[14] Zinc and Copper have been taken up by mycorrhiza in a deficient condition to increase plant yield. Paradoxically, there is evidence that VAM can inhibit Zinc and Manganese (Mn) uptake at toxic concentration in soil thus reducing adverse effect on host.

## 2.2 Importance of arbuscular mycorrhizal fungi in soil ecology

Although plants are important in soil aggregate formation, the role of AMF is as vital. Because AMF symbiosis influences plant physiology such as root-to-shoot ratio, nutrient content, and rhizodeposition, plant effects on soil aggregate formation, to a large extent, are governed by AMF activities. Plants roots are known to exert some pressure on soil particles, thereby aligning and binding the particles together to facilitate soil aggregate formation.[15] AMF contribute to soil Carbon storage, a large fraction of soil Carbon is labile and can be easily decomposed when exposed to microbes, especially under high temperature and moisture. However, when these labile Carbon fractions are stored in soil aggregates, they are better protected and decompose less than when in bulk soil. Generally, all attributes of AMF facilitate carbon storage; while the intraradical mycelium enhances $CO_2$ fixation and rhizo-deposition by plants, the extraradical mycelium promotes the storage of the acquired carbon in aggregates. Additionally, because erosion is a main channel of soil organic carbon (SOC) losses, AMF can reduce carbon lost via erosion through the formation of water stable aggregates. A well structured soil is less susceptible to wind and water erosion compared with a poorly structured soil.[16] Soil organic matter is of great significance in determining or influencing numerous aspects of soil quality, including nutrient storage capacity and water-holding capacity. Thus, AMF are not only a factor but also key determinants of soil quality.

Arbuscular mycorrhizal fungi (AMF) have numerous well-documented effects on plant nutrition. AMF enhance plant water relations through several mechanisms, potentially contributing to increased crop drought resistance.[17] AMF and their product glomalin related soil protein (GRSP) play a decisive role in the soil aggregation, affecting the carbon (C) dynamics in agro ecosystems. Tillage affects the AMF activity and GRSP content, influencing the stability and the soil Carbon forms as well. AMF could interact with beneficial microbes, i.e. phosphate solubilizing bacteria with potential beneficial contributions to nutrient cycling and plant nutrition.[18]

### 2.2.1 Effects of VAM on drought stress

AMF symbiosis protect host plants against detrimental effects caused by drought stress. Drought stress is a major agricultural constraint in the semi-arid tropics.  It is known to have a considerable negative impact on nodule function. Several mechanism have been proposed  to explain the protection of AMF symbiosis, such as changes in plant hormones, increased leaf gas exchange and photosynthetic rate; direct hyphal water uptake from the soil and transfer to the host plant, enhanced activity of enzymes involved in anti-oxidant defence,[19] nitrate assimilation, enhanced water uptake through improved hydraulic conductivity and increasing leaf conductance and photosynthetic activity,[20] osmotic adjustment  and changes in cell-wall elasticity. Often mycorrhizal improvement of drought tolerance occurs via drought avoidance.

### 2.2.2 Restoration of degradated areas using VAM fungi

The soils of disturbed sites are frequently low in available nutrients and lack the nitrogen-fixing bacteria and mycorrhizal fungi usually associated with root rhizospheres. As such, land restoration in semi-arid areas faces a number of constrains related to soil degradation and water shortage. As mycorrhizae may enhance the ability of the plant to scope with water stress situtations associated to nutrient deficiency and drought, mycorrhizal inoculation with suitable fungi has been proposed as a promising tool for improving restoration sucess in semi-arid degraded areas.[21] Degraded soils are common targets of revegetation efforts in the tropics, but they often exhibit low densities of AMF fungi. This may limit the degree of mycorrhizal colonization in transplanted seedlings and consequently hamper their seedling establishment and growth in those areas. Soil inoculation with *G. mosseae* has significantly enhanced plant growth and biomass production in limestone mine spoils.[22]

## 2.3 Soil Organic Matter

Arbuscular Mycorrhiza symbiosis facilitates plant growth through enhancing uptake of several macro- and micro-nutrients of low mobility in soil. AM also contributes to numerous ecological advantages like influencing microbial and chemical environment of the mycorrhizosphere, stabilizing soil aggregates. In the soil, plant-mycorrhizae produced organic carbon are found in two pools: (I) the labile, “light” or particulate organic matter (POM) fraction and (II) the recalcitrant, “heavy” or humic fraction. The Particulate organic matter (POM) fraction represents fresh or partially decomposed plant material, while the humic fraction is more completely decomposed material. Changes in POM concentration are correlated with changes in soil fertility due to tillage practices or environmental factors. As POM degrades further, it is transformed into humic substances. The "heavy" fraction contains three types of humic substances:

(i) Humic acid (HA)
(ii) Fulvic acid (FA)
(iii) Humin

Humic substances are considered important in sustainable agriculture because they enhance water-holding capacity, permeability, soil aggregation, buffering capacity, and cation exchange capacity.[23]

The recently discovery of soil organic matter (SOM) component ‘glomalin’ is a ubiquitous and abundant glycoproteinaceous molecule. However, unlike particulate organic matter (POM) or humic substances, glomalin is not derived from the decomposition of plant or microbially produced material. Glomalin forms a hydrophobic sheath on hyphae that may keep material from being lost from across the hyphal membrane and/or may protect the hyphae from microbial attack. Its presence in soil helps to stabilize aggregates. Glomalin appears to be highly correlated with aggregate stability and with carbon sequestration in the soil by helping to physically protect organic matter within aggregates.[24]

The increased soil organic carbon (SOC) levels are needed to improve crop yields. The benefits of SOC are accepted to be:

- Improved soil structure for root growth and water infiltration
- Increased water holding capacity
- More available nutrients from recycled organic matter.

### 2.3.1 GRSP (Glomalin-related soil protein) is AMF Origin

Glomalin-related soil protein component is produced by arbuscular mycorrhiza, and as stable glue the hyphae has an important role in soil aggregate stabilization. The glomalin produced from some crop rotation cropping system could promote aggregate stability. The quantification of glomalin can be divided into two fractions; first, easily extractable glomalin and second, total glomalin. Both of them show different responses to land use change. Arbuscular mycorrhiza (AM) fungi have been related to aggregate formation and stability. Arbuscular mycorrhizal fungi (AMF) occur in the soil of most ecosystems, including polluted soils. AMF form symbiotic networks with host plant roots, the fungi scavenge nutrients from soils and transfer these nutrients to the host plant in exchange for carbohydrates. Host plants rely upon mycorrhizal fungi to acquire nutrients such as phosphorus and nitrogen for growth. [25]

Glomalin is a yet to be biochemically defined protein measured operationally in soils as glomalin-related soil protein (GRSP). GRSP is relatively long lived in soil, with portions of GRSP likely in the slow turnover soil carbon pool, highlighting the structural role this compound is hypothesized to play in soil carbon dynamics.

Glomalin is primarily hyphal wall-bound, with secretion playing a subordinate role, and then glomalin would likely have primary functionality for the AMF mycelium in the hyphal wall, as opposed to in the soil. Additionally, the effects of GRSP on soil aggregation, and its longevity in the soil, could vary greatly based on the mechanism of entry into the soil (Figure 2.3). Secretion of glomalin into the soil could imply potentially greater mobility in soil, while possibly contributing to faster breakdown through exposure to microorganisms.

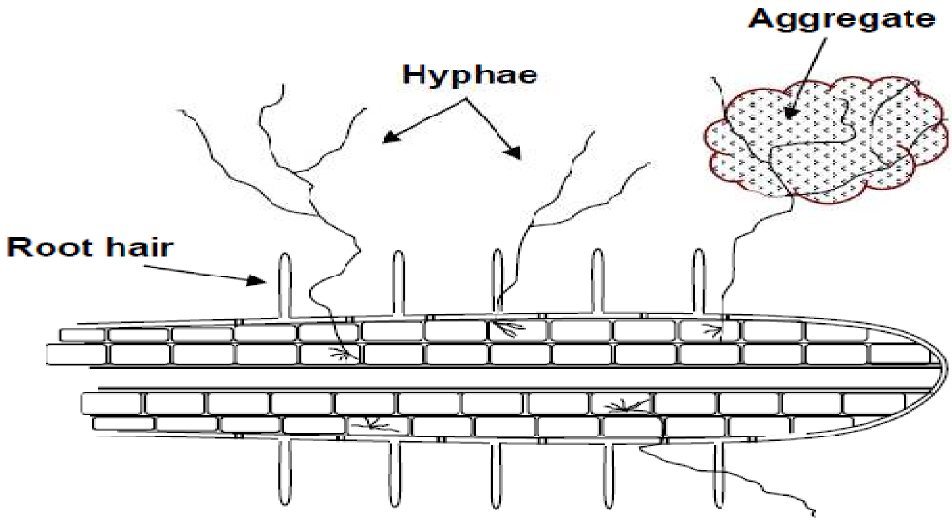


Figure 2.3- Hyphae of AMF form a frame for soil particles to collect into aggregates which are coated with glomalin. [26]

In contrast, incorporation of glomalin into the fungal hyphal wall likely requires subsequent microbial degradation of this complex. Understanding the incorporation and stability of glomalin in the hyphal wall could help explain the relative stability of GRSP in soil.[26] Having a secondary specific detection system would clearly greatly enhance the confidence in the association between GRSP and AMF. However, in the absence of such a system there are still several pieces of evidence that are supportive of the hypothesis that at least some portion of GRSP is of AMF origin.

## 3. GLOMALIN – A GLYCOPROTEIN PRODUCED BY AMF

Soil aggregation is a complex process that is largely dependent upon microorganisms to provide glues that hold soil particles together. AMF are ancient microorganisms that evolved with plants as they moved from water to land. The VAM fungus is beneficial to plants because hyphae, hair-like projections of the fungus, explore more soil than plant roots can reach and transport phosphorus and some other nutrients to the plant. In return, plants provide carbon for growth of the fungus, that glomalin protects hyphae during transport of nutrients from the plant to the hyphal tip and from soil to the plant. When a hypha stops transporting nutrients, that glomalin comes off of the hypha and moves into soil where it attaches to minerals and organic matter (Figure 3.1). The fungus is continually moving down a plant root and forming new hyphae, so individual hyphae is not as important as the whole mass of hyphae that come and go during the life of the plant.

The Glomalin discovery in 1996 by United States Department of Agriculture (USDA), Agricultural Research Service (ARS) soil scientist Sara F. Wright,[27] this soil "super glue" was mistaken for an unidentifiable constituent of soil organic matter. Rather, it permeates organic matter, binding it to silt, sand, and clay particles and its concentratation in soil is strongly positively correlated with the water-stability of soil aggregates.

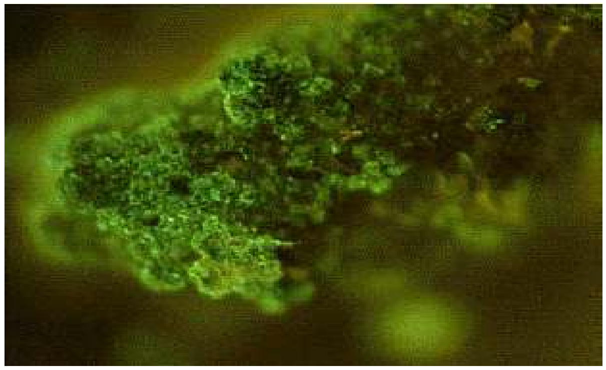

Figure 3.1- Glomalin in its natural state is brown. A laboratory procedure reveals glomalin on soil aggregates as the green material shown here. [27]

Glomalin, a stable and persistent glycoprotein, is released by hyphae and spore arbuscular mycorrhizal fungi in the taxon Glomales, including fungi of the genera *Acaulospora, Entrophospora, Gigaspora, Glomus, and Scutellospora*. Glomalin binds to soil, producing a uniform aggregated structure composed of minerals and humus. Increasing organic matter increases cation exchange capacity of soils. Primarily, these aggregates permit the soil to retain water better and facilitate root penetration. In addition, the aggregates reduce soil erosion and compaction while facilitating root hair adhesion, enhancing nutrient and water uptake. Soil disturbance leads to increased hydrolysis of the glomalin molecule and reduced production of glomalin due to disruption of the network of mycorrhizal hyphae.[25]

Glomalin appears to have properties and functions similar to fungal hydrophobins, which is small, self aggregating, hydrophobic proteins found on hyphae of many types of fungi, including ectomycorrhizal fungi.[28] The glomalin protein some molecular properties contain iron, appear to have N-linked oligosaccharides and are insoluble and possibly hydrophobic in its native state. Soil structure is important for facilitating water infiltration, biogeochemical cycling processes, resistance against erosional soil loss, and soil carbon storage.[29] Glomalin is a stable compound, insoluble in water and resistant to heat degradation. Because it is a glue like in nature attaches to horticultural film and soil surfaces, glomalin is likely hydrophobic in its native state. Apart from the Glomeromycota, no other fungal group produces this glycoprotein in significant amounts. Glomalin is found in agricultural, grassland, forest, desert and non-cultivated soils.[27]

Glomalin, through still not biochemically defined, is an N-linked glycoprotein composed of 3 to 5% N, 36 to 59% C, 4 to 6% Hydrogen, 33 to 49% Oxygen, and 0.03 to 0.1% P. Glomalin also contains 0.8 to 8.8% Fe, which may be responsible for the reddish color of glomalin extracts and to soil structure stabilization as a biochemical binding agent in soil particle aggregation (GRSP). Glomalin accumulates in soils is thought to result from the insolubility, hydrophobicity and high Fe content of the molecule. Iron concentrations of 0.8 to 8.8% protect glomalin from degradation by as proposed for the role of Fe in organic matter and may increase the thermal stability and antimicrobial properties of glomalin.[30]

### 3.1 Identification of Glomalin

The identification of glomalin, a glycoprotein produced by arbuscular mycorrhizal fungi with lead to a reevaluation of fungal contributions to soil organic matter (SOM) and aggregate stability. Glomalin was identified at the States Department of Agriculture (USDA) in the early 1990's during work to produce monoclonal antibodies reactive with AM fungi. One of these antibodies reacted with a substance on hyphae of a number of AM species. This substance was named glomalin after *Glomales*. The glomalin fraction is operationally defined by its extraction procedure but is further characterized by total and immunoreactive protein assays.[31]

The glomalin identification is base on solubility characteristics: (i) easily extractable glomalin (EEG), (ii) total glomalin (TG) and (iii) a'scum'. This 'scum' is apparently a sloughed component of glomalin and is very hydrophobic.[32] Typically, glomalin concentration in these pools is measured by a Bradford total protein assay (i.e. TG and EEG) and immunoreactive protein assays. The Bradford protein assay is non-specific and detect any proteinaceous material. The immunoreactive protein assay, or enzyme-linked immunosorbent assay (ELISA), uses the monoclonal antibody specific for glomalin, but certain artificial conditions may reduce immunoreactivity.


Figure 3.2- Glomalin is extracted from soil with high heat. After removal of glomalin, soil is transformed from a rich brown color to a grey mineral color.[33]

In the soil, organic matter, metals (such as iron), clay minerals, and other substances may bind to glomalin causing conformational changes or masking the reactive site. Glomalin is dark red-brown color and soil after extraction loses the brown color associated with organic matter (Figure 3.2). The brown color of glomalin was hypothesized to be due to incorporation of iron as a structural component and may play a role in accumulation and/or function.[33]

The unusual extraction conditions remove high quantities of the rich organic material (i.e. glomalin) leaving soil a mineral grey color. Glomalin accounts for a large amount (about 15 to 20%) of the organic carbon in undisturbed soils. Glomalin accumulation of external hyphae, auxiliary cells, spores, or internal structures (intraradical hyphae, arbuscules, vesicles) and soil aggregation. The correlation between glomalin concentration and soil aggregation also may be influenced by iron. Iron- and Al-(hydr) oxides are speculated to be involved in aggregate formation by bridging organic matter to clay minerals and to contribute to the persistence of aggregates.

## 3.2 Glomalin Extraction

Glomalin-related soil protein is extract from field soil, roots, mesh (horticultural or nylon) strips or bags, or pot culture media (sand or crushed coal). The extract solution then used in further analyses (e.g. ELISA, Bradford total protein assay and dot blot assay). Caution must be used in the current analysis of glomalin since the extraction protocol may co-extract other soil proteins and humic substances-[25]

**Table 3.1** Current terminologies for glomalin extraction and their definitions

| **Current terminologies for glomalin and their definitions (modified from Rillig, 2004b). Terminology** | **Description** |
|---|---|
| 1. Glomalin | A yet to be identified putative gene product of arbuscular mycorrhizal fungi |
| 2. Glomalin-related soil protein (GRSP) | Total soil glomalin fraction, possibly contains other soil protein; fraction of soil glomalin extracted repeatedly using 50 m*M* sodium citrate solution (pH 8) and autoclaving at 121˚C for 60 min until glomalin extract is straw-coloured |

| | |
|---|---|
| 3. Easily extractable glomalin-related soil protein (EE-GRSP) | Fraction of soil glomalin extracted once using 20 m*M* sodium citrate solution (pH 7) and autoclaving at 121˚C for 30 min |
| 4. Bradford-reactive soil protein (BRSP) | Glomalin-related soil protein quantified using the Bradford assay, measures all protein in glomalin extract |
| 5. Easily extractable Bradford-reactive soil protein (EE-BRSP) | Easily extractable glomalin-related soil protein quantified using the Bradford assay, measures all protein in glomalin extract |
| 6. Immunoreactive soil protein (IRSP) | Glomalin-related soil protein quantified using an indirect enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody MAb32B11, specific for glomalin, though may cross-react with other soil protein |
| 7. Easily extractable immunoreactive soil protein (EE-IRSP) | Easily extractable glomalin-related soil protein quantified using an indirect enzyme-linked immunosorbent assay (ELISA) with monoclonal antibody MAb32B11 |

### 3.2.1 Compositional Analysis of Glomalin

Proteins are the most complex naturally occurring macromolecules. Glycoprotein's act in enzyme catalysis, hormonal control, immunology, ion transport, structural support, cell adhesion, and cell recognition. Carbohydrates affect viscosity, thermal stability, solubility, and resistance to proteolysis.[34] Glomalin is resistant to trypsin and chemical (acid) hydrolysis. Lectin-binding capability and high performance capillary electrophoresis (HPCE) indicate that glomalin is a glycoprotein with one major asparagine-linked (N-linked) chain of carbohydrates. In its native state, glomalin is insoluble in aqueous solutions. High heat (121$^{o}$C) treatment in one hrs intervals is used to solubilize glomalin. These denatured proteins and other small molecules are lost during the primary purification process of acid precipitation, re-dissolution in an alkaline solution and dialysis. Because glomalin is so resistant to decomposition, it is a fraction of organic matter (OM) that may be present in both the transient and persistent pools with a turnover time of at least a decade. Molecular stability from chemical characteristics, such as hydrophobicity and iron binding. Hydrophobicity makes glomalin water-insoluble, prevents microbial access to the molecule, and helps it bind to surfaces. Iron-binding prevents microbial decomposition and bridges glomalin to clay minerals and other types of organic matter. Iron may also act as a bridge between clay minerals and glomalin. This compositional groups of glomalin from hyphae, soil extracts and its importance in global climate change and soil fertility issues.[32]

### 3.3 Function of 'Glomalin' in soil

Arbuscular mycorrhizal (AM) fungi are key organisms of the soil/plant system, fundamental for soil fertility and plant nutrition. Arbuscular mycorrhizal fungi, found living on plant roots around the world, appear to be the only producers of glomalin. These mutualistic fungi use carbon from the plant for their growth, and in return provide water and nutrients, particularly phosphorus, to the plant through their hair like hyphae which extend beyond the absorption surface and reach that can be achieved by the plant's roots. Glomalin is detectable on the surface of these hyphae, and is believed to provide the rigidity required by the hyphae to grow into the air spaces between soil particles. When older hyphae stop transporting nutrients, their protective glomalin coating is sloughed off into the soil, where it attaches to mineral particles and organic matter, forming micro aggregates. By contrast 'glomalin' arises from a variety of recalcitrant soil proteins, including glomalin.

Glomalin is believed to give soil its tilth, the characteristic which provides soil with good texture and easy of cultivation. This fungi use carbon from the plant to grow and make glomalin. The glomalin attaches to particles of minerals (sand, silt, and clay) and organic matter, forming clumps. This type of soil structure is stable enough to resist wind and water erosion, but porous enough to let air, water, and roots move through it.

Glomalin is extremely "tough". It is resistant to microbial decay (lasting at least 10 to 50 years) and does not dissolve easily in water. These properties make glomalin a good protector of hyphae and soil aggregates.[35] It is therefore likely, although untested in dry land conditions, that agricultural management which sustains 'glomalin' and glomalin in crop and fallow will lead to the benefits of improved tillage, soil organic carbon, enhanced nutrient content, erosion prevention and ultimately more stable and sustainable systems. It is found in soil and climate conditions that 'glomalin' levels are manageable through different agricultural practices, such as minimum tillage, cover crops, reducing phosphorus inputs, and a reduction in the use or distribution of non AM crops, which primarily constitutes the *Brassica* family. Although fallow systems have never been tested, it is likely that improved fallows in particular could similarly be used to increase soil 'glomalin' levels.

## 4. SOIL AGGREGATION

Aggregation processes in soil are influenced by a large number of factors such as changes in soil organic matter (SOM), moisture content and microbial activity, crop type root development, tillage and fertilization. The aggregate as a "naturally occurring cluster or group of soil particles in which the forces holding the particles together are much stronger than the forces between adjacent aggregates".[36] The structural stability is dependent on particle size distribution, soil organic matter, vegetation and soil micro-organisms and its stability is influenced by exchangeable cations. One of the most important binding agents for forming stable aggregates is soil organic matter (glomalin). Organic materials are important soil additives to improve soil physical properties.[37]

Aggregate formation is a complex process of physical and chemical interactions. Soil aggregates results from a combination of primary mineral particles with organic and inorganic materials. This process, dynamic and complex, is influenced in turn by the interaction of several factors including environmental components, soil management, plant effects but largely by soil properties. Soil structure is often expressed as the degree of stability of aggregates being a major factor which moderates physical, chemical, and biological processes leading the soil dynamics. All the major factors playing a role in aggregate formation and stabilization, the following factors influenced soil aggregation:- (1) soil fauna, (2) soil microorganisms, (3) roots, (4) inorganic binding agent (like glomalin), (5) environmental variables (Figure 4.1). Soil aggregates are a conglomeration of soil minerals (clay particles, fine sand and silt), small plant or microbial debris, bacteria, organic matter strongly associated with clay coatings.[38]

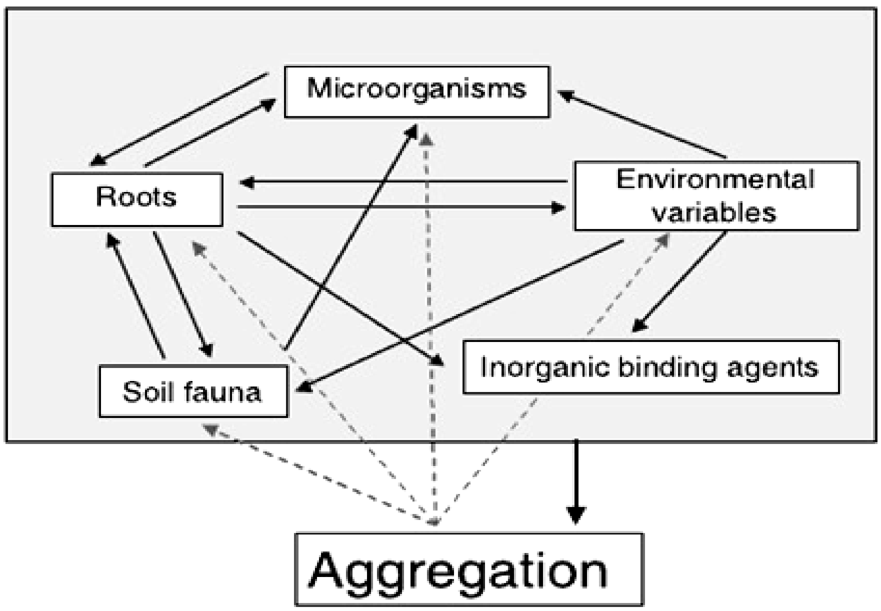


Figure 4.1- The multiplicity of interactions and feedbacks between the five major factors influencing aggregate formation and stabilization.[38]

Glomalin contributes to the stabilization of aggregates by sloughing off hyphae onto the surrounding organic matter, binding to clays (via cation bridging by iron), and providing a hydrophobic coating. This is demonstrated in a number of experiments, where total and, especially, immunoreactive concentration of glomalin are positively correlated with percent water-stable soil aggregates in both agricultural and native soils. [39, 40] Hyphae act as a frame upon which soil particles collect while glomalin glues them together and protects them. This is similar to walls in a house, where boards (i.e. hyphae) are used to frame-up the wall, insulation (i.e. soil particles) fills in spaces between boards, wall board (i.e. microbial glues, like glomalin and fungal and bacterial polysaccharides) help keep everything in place, and finally it is all coated with a protective layer of paint (i.e. glomalin). Sticking soil particles together (i.e. aggregate formation) is just one part of the process and one role for glomalin and other microbial polysaccharides. Glomalin is an important molecule in aggregate stabilization. When aggregates are not stabilized, they break apart with rainfall. Organic matter and nutrients within disrupted aggregates may be lost to rain and wind erosion. The chemistry of glomalin makes it an ideal stabilizing coat.

They increase the contact angle for water penetration, which restricts infiltration and slaking, lowers wet ability and increases the internal cohesion of aggregates. The Soil aggregates most important strategies proposed for maintaining and improving soil fertility are those which target the physical properties of the soil. The abundance and stability of the aggregates are critical for several soil functions- Maintaining soil porosity, which provides aeration and water infiltration rates favorable for plant and microbial growth, increasing stability against wind and water erosion, and storing carbon by protecting organic matter from microbial decomposition.[33]

### 4.1 GRSP Relationship with Soil Aggregate Water Stability

It is important to appreciate that the relationship between glomalin related soil aggregation (GRSP) and soil aggregate water stability is a large range of water stabilities. This means that beyond a certain "saturation" GRSP concentration in a given soil, additional deposition of GRSP will not result in detectable increases in soil aggregate water stability, at least as measured with the conventional disintegrating forces.[41] The curvilinear pattern of aggregation of soils with high GRSP concentrations fairly saturated with GRSP, perhaps because most pores in these macro-aggregates have already been partially "sealed" by deposition of this substance, slowing down penetration of water into the aggregate. This relationship of GRSP with soil aggregate water stability applies only to hierarchically structured soils, in which organic material is the main binding agent. In a soil in which carbonates are the main binding agent, none of the GRSP fractions are positively correlated with aggregate stability.[42]

### 4.2 Advantages of Glomalin

The fungi live on most plant roots and use the plants' carbon to produce glomalin. Glomalin is thought to seal and solidify the outside of the fungi's pipe like filaments that transport water and nutrients to plants. As the roots grow, glomalin sloughs off into the soil where it acts as "super glue," helping sand, silt and clay particles stick to each other and to the organic matter that brings soil to life. It is glomalin that helps give good soil, as smooth clumps of the glued-together particles and organic matter.

Glomalin is long lost in humus, the organic matter that is often called "black gold." It also provides nitrogen to soil and gives it the structure needed to hold water and for proper aeration, movement of plant roots and stability to resist erosion-

- Beneficial to most crop plant's
- Found in all soils

- Produced in large amounts
- Extremely “tough”
- Does not dissolve in water
- Resistant to decay

#### 4.2.1 Biofertilizers and Nutrients

The glomalin protein primary effect on improved nutrients management in soil are increase plant productivity, soil organic carbon (SOC) and biological activity. Increase in SOC by biofertilizers use increases aggregation.[43] Biofertilizer use also improves residue quality and quantity, but this does not necessarily increase SOC pool. Biofertilizers applications alter soil pH and the electrolyte concentrations in soil, which can have adverse effects on soil structure. The beneficial effects of biofertilizer applications generally offset any adverse affects of fertilization. Increases in plant residues and below-ground plant growth increase calcium (Cl), microbial activity, which improve aggregate stability. [44]

**Function**

- Protect hyphae from nutrient loss
- Glue together soil aggregates
- Stabilize aggregates
- Reduces wind and water erosion
- Increases water infiltration
- Increases water retention near roots
- Improves nutrient cycling
- Improves root penetration by reducing compaction
- Soil carbon and/or nitrogen storage

### 4.3 Management of glomalin in soils

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.[45]

- Minimum or no-till to reduce disruption of hyphal network
- Cover crops to maintain living roots
- Reduced inputs, minimum Phosphorus

- 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.

- Use cover crops to maintain living roots for the fungi to colonize.
- Maintain adequate phosphorus level for crops, but does not over-apply P because high levels depress the activity of these fungi.

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