THE ROLE OF GLOMALIN IN SOIL EROSION

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Abstract: Glomalin is a glycoprotein, a sugar protein compound that might trigger the formation of soil. In this study we analyze the different organic matters which inhance mycorrhizal fungi and produce glomalin which is dependent upon the types of organic matter. The more glomalin in a particular soil, the soil probably is better. The amount of glomalin in the soil increased as a degree of interdependence increased between plants and arbuscular mycorrhizal fungi. These fungi produce glomalin and live inside plant roots and in the surrounding soil.

Growth of Arbuscular mycorrhizal fungi under field conditions was estimated within growth mesh bags which contain different organic matter. After six months these mesh bags were harvested. These soil were analysed in Montana University, USA by two detection methods utilized to quantify Glomalin related soil protein (GRSP): Bradford protein assay, yielding Bradford reactive soil protein (BRSP), and an enzyme-linked immunosorbent assay (ELISA: using the monoclonal antibody Mab32B11 developed against crushed spores of *Glomus intraradices*,yielding the immunoreactive soil protein. The amount of GRSP in the mesh bags was positively related to organic matter addition. Furthermore, GRSP content was positively correlated to NLFA 16:1ù5 as well as to PLFA 16:1ù5 and bacterial PLFAs .In contrast no correlation was found between spore number and neither fatty acids nor GRSP.

Key words : Organic matters; Arbuscular mycorrhizal fungi; Glomalin; Climate change; Green house gas.

INTRODUCTION:

The study of glomalin started out with a monoclonal antibody (MAb32B11) raised against an unknown epitope on crushed spores of the AMF species *Glomus intraradices* (Wright and Upadhyaya 1996). This monoclonal antibody reaction has been used to operationally define glomalin. This in itself is not unusual, as it is common practice in soil science to define fractions of organic matter (such as humic acids, fulvic acids, humin) by their solubility/ extractability and or a variety of other physico-chemical properties. The case of glomalin is no different in principle, since glomalin is defined via extraction conditions from soil (citric acid buffer, autoclaving, a PH of either 7.0 or 8.0) and its antibody reaction (with MAb32B11). However, it is clear that, from the beginning, it was hypothesized that glomalin is a specific protein (or group of proteins).

Glomalin is a glycoprotein, a sugar protein compound that might trigger the formation of soil. The more glomalin in a particular soil, the soil probably is better. The amount of glomalin in the soil increased as a degree of interdependence increased between plants and arbuscular mycorrhizal fungi. These fungi produce glomalin and live inside plant roots and in the surrounding soil. The fungi have hair like filaments called ' hyphae' that extend the reach of plant roots.

Farmer's survey conducted in the western hills of Nepal identified erosion as the primary cause of soil fertility decline. The three major factors (soil losses by erosion, inefficient use of existing nutrients both organic and inorganic nutrients inputs) were recognized for declining soil fertility resulting in low agricultural productivity in the western hills of Nepal (Tripathi *et al.* 1997). Decline in soil fertility of 25-30% over the last 20 years was reported by farmers in the Mid/High hills (Subedi *et al.* 1989).

The quantity of chemical fertilizer used per hectare in Nepal is very low as compared to other countries. However farmers who have been using chemical fertilizer in Kathmandu valley and some of the Terai districts have started to experience its adverse effects on soil quality. Soils of Nepal are deficient in N, P, and K due to shortage of organic matter in the soils. Therefore incorporation of organic matter is necessary for improving soil fertility. This organic matter replaces the use of chemical fertilizers as much as possible which will improve the environmental quality.

Organic amendments enhanced spore production of AM

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Table 1: Content of GRSP fractions in mesh bags collected from *Bauhinia purpurea* trees

Treatment	EE-BRSP (mg g^{-1} soil)	BRSP (mg g^{-1} soil)	EE-IRSP (mg g^{-1} soil)	IRSP (mg g^{-1} soil)
Control	0.08±0.01	0.2±0.03	0.15±0.05	0.2±0.08
Lantana camara	0. 7±0.2	1.4±0.3	0. 7±0.4	1.9±0.5
Tithonia diversifolia	0.7±0.3	1.2±0.4	0.5±0.2	0.6±0.3

(For explanations of different forms of glomalin see Rillig 2004; BRSP = Bradford-reactive soil protein, IRSP = MAb32B11-immunoreactive soil protein, EE = easily extractable)

mycorrhiza (Johnson and Mc Graw 1988; Douds *et al.* 1997; Shrestha Vaidya *et al.* 2008). Organic matter addition to the soil in eroded sites could thus be an appropriate method to enhance the beneficial effect of AM fungi on soil stabilization and plant establishment and it also protects environment over the long term and reducing costs of production. This is mainly because organic farmers do not use inorganic N fertilizers. Soil contains about twice as much carbon as the atmosphere. The use of inorganic fertilizers and higher production levels in agriculture may have caused a large loss in organic matter from the soils.

The quantity of chemical fertilizer used per hectare in Nepal is very low as compared to other countries, but farmers regularly using chemical fertilizer in Kathmandu valley and some of the Terai districts have started to experience its adverse effects on soil quality. Soils in Nepal are deficient in N, P and K due to shortage of organic matter in the soils. This organic matter replaces the use of chemical fertilizers as much as possible which will improve the environment quality. Application of organic matter is essential to maintain both soil fertility, soil structure, and to stimulate extensively biological activity and enhance arbuscular mycorrhizal fungi. Numerous studies have shown that CO_2 emissions from organic farming are 40 – 60 % lower per hectare than conventional systems. This is mainly because organic farmers do not use inorganic N fertilizers. Soil contains about twice as much carbon as the atmosphere. Glomalin contains approximately. 30- 40% of carbon and forms small soil clumps. This granulated material mellows the soil and binds carbon in the ground.

MATERIALS AND METHODS

Growth of arbuscular mycorrhizal fungi under field conditions was estimated within growth mesh bags (Shrestha vaidya *et. al*. 2008). After six months these mesh bags were harvested and soils were sent to Prof. Rillig Mathias of Montana University, USA for glomalin analysis.

Glomalin related soil protein (GRSP) analysis:

There are currently two detection methods utilized to quantify Glomalin related soil protein (GRSP): Bradford protein assay, yielding Bradford reactive soil protein (BRSP), and an enzyme-linked immunosorbent assay (ELISA: using the monoclonal antibody Mab32B11 developed against crushed spores of *Glomus intraradices* (Wright and Upadhyaya 1998), yielding the immunoreactive soil protein (IRSP). The first step in the extraction process is to recover the EE-BRSP and EE-IRSP soil fraction (EE = easily extractable). This was done by autoclaving 1.0 g of soil with 20 mM sodium citrate, pH 7.0 at 121°C for 30 minutes. Only one autoclave cycle is required to obtain this fraction. Following this extraction process, the BRSP and IRSP fractions were extracted from the same soil sample using 50 mM sodium citrate, pH 8.0 and repeated autoclaving at 121°C for 60 min. After each extraction/ autoclaving cycle the sample was centrifuged at 5000 x g for 15 min. The supernatant was decanted and stored at 4°C until analysis. The extraction process continued until the supernatant is clear/ light yellow in color. Once the extraction process was complete each extract was centrifuged at 10,000 x g. The Bradford assay was first utilized to determine the concentration of EE-BRSP and BRSP using bovine serum albumin as a standard. Immunoreactive protein values were measured using an indirect ELISA with MAb32B11 (Wright and Upadahyaya 1998). GRSP was only analyzed in a subset of samples for two litter types and the control without litter to explore the relationship with other variables.

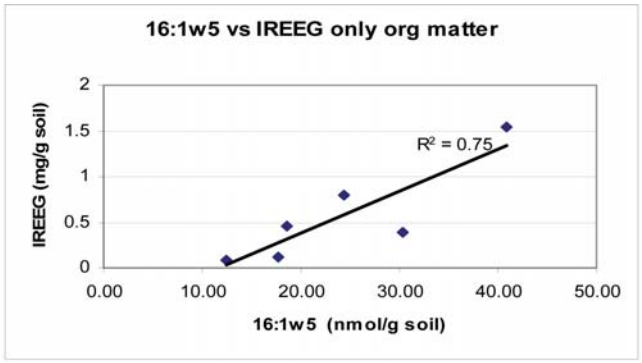
Total (glomalin) protein was determined with a Bradford assay, using BSA as standard. Thus, four fractions of glomalin were measured: EEG, TG, IREEG, and IRTG. Concentration of glomalin was extrapolated to mg/g by correcting for the dry weight of coarse fragments (>0.25 mm) included in the extraction of soil (Rillig *et al.* 2002).

RESULTS

The amount of GRSP in the mesh bags was positively related to organic matter addition (Table 1). Furthermore, GRSP content was positively correlated to NLFA 16:1ù5 as well as to PLFA 16:1ù5 and bacterial PLFAs (Fig. 1).In contrast no correlation was found between spore number and either fatty acids nor GRSP.

The mesh bags had the following amendments: control (no amendment), dried leaves of *Tithonia diversifolia* and *Lantana camara*.

The PCA analysis of the PLFA data indicated that the composition of the microbial communities differed between mesh bags amended with dry leaves from *Eupatorium* and *Lantana* and compost while mesh bags amended with rock phosphate, *Tithonia* or had no amendment (control) could not be separated based on the PLFA pattern (Fig.2). Mesh bags amended with *Eupatorium*, and *Tithonia* were clearly separated from the other mesh bags along PC1 (Fig.2) and the most important PLFA separating these mesh bags from the other ones was the Gram negative PLFA cy 19 which increased in proportion in the *Eupatorium adenophorum* and *Lantana camara* treatments.



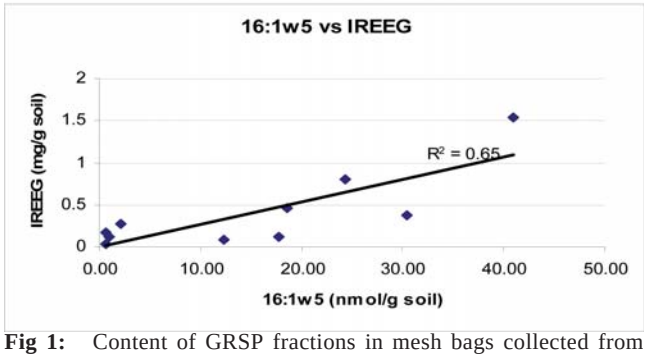
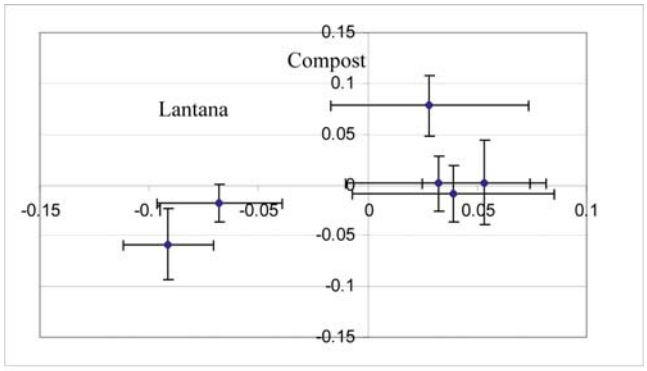


Fig 1: Content of GRSP fractions in mesh bags collected from *Bauhinia purpurea* trees (between June 2003 and December 2003). (For explanations of different forms of glomalin see Rillig 2004; BRSP = Bradford-reactive soil protein, IRSP = MAb32B11-immunoreactive soil protein, EE = easily extractable).



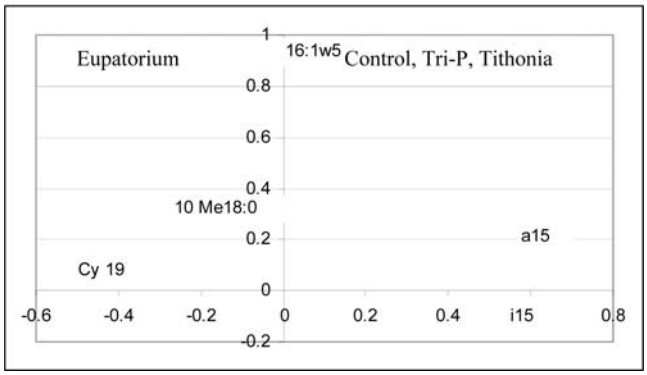


Fig 2: The phospholipid fatty acid patterns in mesh bags buried around *Leuceania diversifolia* and *Bauhinia purpurea* trees during the wet season.

DISCUSSION:

The mesh bag method also made it possible to quantify yield of GRSP (Glomalin related soil protein) under field conditions. GRSP concentration is related to stability of aggregates in soil (Rillig 2004), and thus small increases in this compound are particularly important to eroded soil of low aggregate Water stability is linear, whereas this relationship plateaus at higher stability levels (Wright and Upadhyaya 1998).

The addition of compost or green manure is an important way to improve the soil in degraded areas since nitrogen and other nutrients, as well as organic matter which improves soil structure, is added with the organic material (Caravaca *et al.* 2002; Muthukumar and Udaiyan 2000, Nziguheba *et al.* 2000). Furthermore, the soil structure may also improve through the increased production of GRSP by AM fungi associated with the plants in the area; this will increase the amount of water stable aggregates in the soil. A higher production of GRSP will also contribute to long-term carbon storage in soils (Rillig 2004), which is especially important in soils with low organic matter content, such as the eroded slopes of Nepal. The amount of fatty acids, including NLFA 16:1ù5, PLFA 16:1ù5 and total bacterial PLFAs was positively correlated with GRSP. The correlation with total GRSP must be interpreted with caution since it has recently been shown that the assay can register other protein sources originating from litter such as the organic material added to the mesh bag in the present study (Rosier *et al.* 2006). However, the positive correlations between fatty acids with the IR fractions of GRSP are more reliable. It has earlier been shown that GRSP is mostly contained in hyphal wall structures (Driver *et al.* 2005). The positive correlation between GRSP and 16:1ù5 while the absence of correlation between spore numbers or spore volumes also suggest that the GRSP in the present study originates from hyphae rather than from spores. However, other data contradict this since the growth rate of AM mycelium was negatively correlated with GRSP concentrations in studies by Rillig and Steinberg (2002) and Lovelock *et al.* (2004). It has been suggested that GRSP may not be a good indicator of AMF biomass/ growth, because rates of production differ among species, and the turnover time is too long, and it may be under strong physiological control (Rillig 2004). This relationship of GRSP with soil aggregate water stability only applies to hierarchially 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 were positively correlated with aggregate stability (Rillig *et al.* 2003). In present experiment the aggregate was found correlated with organic matter and no aggregation was found in control polypropylene experiment. Rillig *et al.* (2002) found increased presence of mycorrhizal fungi in roots and soil, higher soil glomalin concentrations, and increased soil aggregate water stability. Rillig *et al.* (2004) significantly extended such findings by showing that AMF-host species combinations also differentially control the percentage of water-stable soil aggregates, and thus another major ecosystem state variable (i.e. soil structure). Similarly, Rillig *et al.* (2002) used path analysis to show that AMF hyphae and their products (glomalin) were significant contributors to soil aggregate water stability.

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