

## **Glomalin related soil protein (GRSP) content and their relationship with structure stability of soil aggregates from Carlos Casares, Argentina.**

Esteban W. Ferrero Holtz; Lidia Giuffr ; Esteban Ciarlo & Gisela Moreno  
*University of Buenos Aires, Faculty of Agronomy, Department of Natural Resources and Environment  
Edaphology. Av. San Mart n 4453. 1417 Buenos Aires. Argentina.  
Corresponding author: Esteban W. Ferrero Holtz*

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**Abstract:** *The large network of fungal hyphae from arbuscular mycorrhizal fungi which spreads from mycorrhizal roots into the surrounding soil, affects the physico-chemical characteristics of soils and represents stabilizing agents in the formation and maintenance of soil structure. The objective of this work was to evaluate the glomalin related soil protein (GRSP) content, the GRSP / Organic carbon ratio (GRSP / Cox) and soil aggregates mean weight diameter (MWD). A mensurative test was carried out in Carlos Casares, province of Buenos Aires, Argentina, with sixteen Entic Hapludoll soils used in livestock production. The content of GRSP was positively correlated with the content of Cox ( $R^2 = 0.60$ ). GRSP / Cox ratio decreases as Cox increases ( $R^2 = 0.71$ ), increasing the recalcitrant carbon proportion (GRSP) as the soil carbon decreases. The MWD of soil aggregates correlated positively with the GRSP content ( $R^2 = 0.59$ ).*

**Keywords:** *Entic Hapludolls, mean weight diameter (MWD), glomalin, arbuscular mycorrhizal fungi*

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### **I. Introduction**

Arbuscular mycorrhizal fungi (AMF) are mutualistic symbionts living in association with the roots of the majority of terrestrial plants. They are key organisms of the soil/plant system, influencing soil fertility and plant nutrition (Smith and Read, 2008).

Soil aggregation supports soil fertility as it reduces erosion and increases soil aeration, and water infiltration and retention (Oades, 1984). Aggregation is mediated by soil organic matter, biota, ionic bridging, clay, and carbonates (Bronick and Lal, 2005).

The large network of fungal hyphae from mycorrhizal fungi arbuscular, which spreads from mycorrhizal roots into the surrounding soil, affects the physico-chemical characteristics of soils and represents stabilizing agents in the formation and maintenance of soil structure (Miller and Jastrow, 2000). More recently, also the heat-stable soil protein called glomalin, which is produced by arbuscular mycorrhizal fungi (AMF), has been shown to cause soil aggregation (Bedini et al., 2009; Harner et al., 2004). A strong relationship between glomalin concentration and the amount of water stable aggregates has been demonstrated (Rillig et al., 2001; Rillig, 2004).

In the soil humus there are fractions composed of proteins that come from diverse microbiological origins, but until now the simple separation and quantification of glomalin from other humic pseudoproteins, has not been possible. The process of extracting glomalin (Wright et al., 2006), derives the use of the term GRSP (glomalin related soil protein) to refer to the generic product of the pool of extracted and quantified soil proteins. This measurement is widely used in AMF studies at a global scale. The objective of this work was to evaluate the glomalin related soil protein (GRSP) content, its relationship with organic carbon (GRSP / Cox Ratio) and with the stability of soil aggregates, measured as their mean weight diameter (MWD), at sixteen Entic hapludoll soils used for livestock production.

### **II. Materials and methods**

A mensurative test was carried out in Carlos Casares, province of Buenos Aires, Argentina, with sixteen soils located in different environments used in livestock production, representing 16 sampling units. These environments, where soil samples were taken, were natural grasslands, monophytic pastures of grasses, and mixed pastures with grasses and leguminous plants (Table 1).

The taxonomic classification of the soil series of the 16 sampling units corresponds to Entic Hapludoll, coarse loam, mixed, thermal family (USDA-Keys to Soil Taxonomy, 2006), it is a marginal soil for agriculture,

and therefore used for cattle raising. Samples were taken at a depth of 0-20 cm, using a shovel, carefully extracting intact and representative aggregates from the surface soil structure.

Soil samples were conducted to laboratory, dried at 40°C for five days and subjected to the following determinations: soil oxidizable carbon (Cox) (Walkley and Black, 1934); extraction and quantification of soil proteins related to glomalin (GRSP) by bicinchoninic acid (BCA) (Stoscheck, 1990); mean weight diameter (MWD) of soil aggregates (Le Bissonnais, 2002); pH at a soil water ratio 1 / 2.5; saturated electrical conductivity by potentiometry, and soil texture by a modified hydrometric method (Bouyocous, 1962). The soils corresponding to samples 3, 4 and 7 (grasslands) presented massive structure (lack of structure) when subjected to the Le Bissonnais test; this weak structure corresponds to natricHalomorphic soils, due to the accumulation of soluble sodium salts, and for this reason the MWD of these soils could not be evaluated.

### Statistical procedures

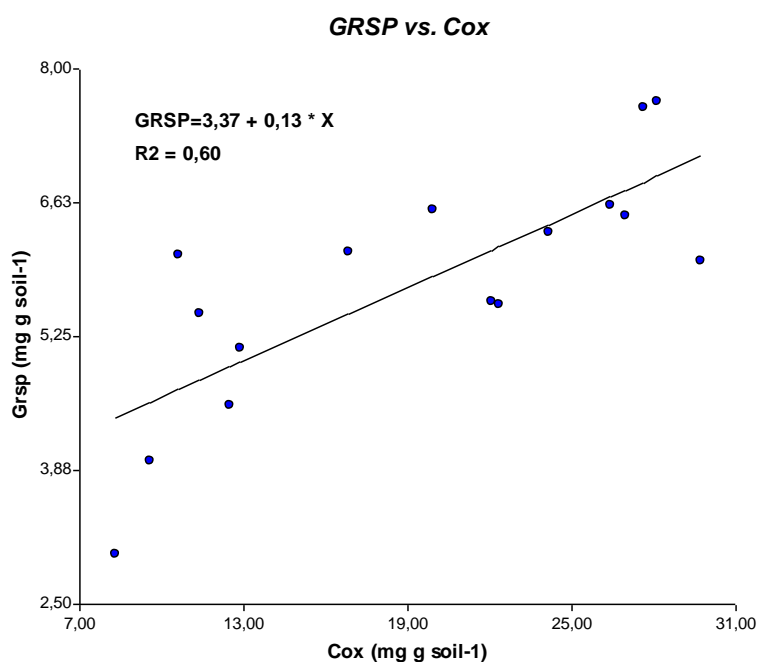
Response variables were GRSP, GRSP / Cox ratio, and structural stability of the soil aggregates, expressed by the mean weight diameter (MWD), performing simple regression analysis to evaluate the degree of association between measured variables, through the InfoStat statistical package.

**Table 1.** Characterization of soil samples

Sample	Position	Vegetation	pH	Ce (ds m <sup>-1</sup> )	Texture
1	Hill	<i>Festucaarundinacea</i> + <i>Lotus tenuis</i>	5,6	0,17	Loamysand
2	Hill	<i>Festucaarundinacea</i> + <i>Lotus tenuis</i>	5,8	0,34	Loamysand
3	1/2 hill	Grassland	8,86	0,75	Loamysand
4	1/2 hill	Grassland	8,3	1,22	Loamysand
5	1/2 hill	<i>Festucaarundinacea</i>	7,5	1,12	Loamysand
6	Low	Grassland	7,8	1,14	Loamysand
7	Low	Grassland	8,6	1,35	Sandy loam
8	Hill	Grassland	7,98	0,62	Loamysand / Sandy loam
9	1/2 hill	Grassland	5,4	0,71	Loamysand
10	Hill	<i>Festucaarundinacea</i>	5,57	0,62	Sandy loam
11	Hill	<i>Festucaarundinacea</i>	6,2	0,74	Loamysand / Sandy loam
12	1/2 hill	Grassland	7,8	1,52	Sandy loam
13	Hill	Grassland	6,15	0,35	Loamysand
14	Hill	<i>Festucaarundinacea</i>	5,64	0,20	Sandy loam
15	Hill	<i>Festucaarundinacea</i> + <i>Trifoliumrepens</i>	5,31	0,31	Sandy loam
16	1/2 hill	<i>Festucaarundinacea</i>	4,8	0,63	Sandy loam

### III. Results and Discussion

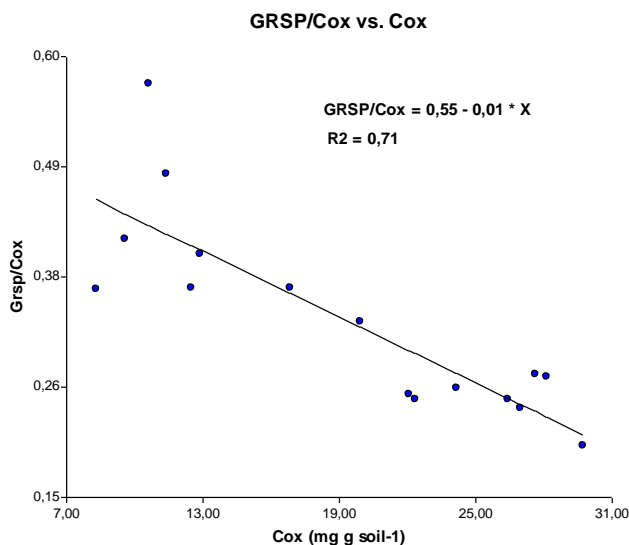
The GRSP contents were related to the oxidizable carbon (Cox) of the soil ( $p < 0.05$ ). A positive linear correlation was observed between GRSP and Cox, with  $R^2 = 0.60$ . The relationship between GRSP and soil carbon has recently been documented (Ferrero Holtz et al., 2018, Kumar et al., 2018, Ferrero Holtz et al., 2016, Nobre et al., 2015) and, according to these previous studies, we confirm that there is a strong positive correlation between GRSP groups and soil carbon (Figure 1). Singh et al. (2016) propose that the factors involved in carbon accumulation simultaneously encourage the proliferation of AMF and the enrichment of GRSP.



**Figure 1.** GRSP (mg g soil-1) as a function of Cox (mg g soil-1). The points represent all the test data.

The proportions of GRSP within organic carbon, measured through the GRSP/Cox ratio, were related to the soil Cox contents ( $p < 0.05$ ). Within the range of Cox explored in this mensurative test (0.7% to 3.1%) the GRSP/Cox values had a negative linear behavior with respect to Cox ( $R^2 = 0.71$ ) (Figure 2). This association agrees with other reports, at agricultural and livestock soils, in the existence of a negative linear association between GRSP/Cox ratio and Cox (Ferrero Holtz et al., 2016, 2018).

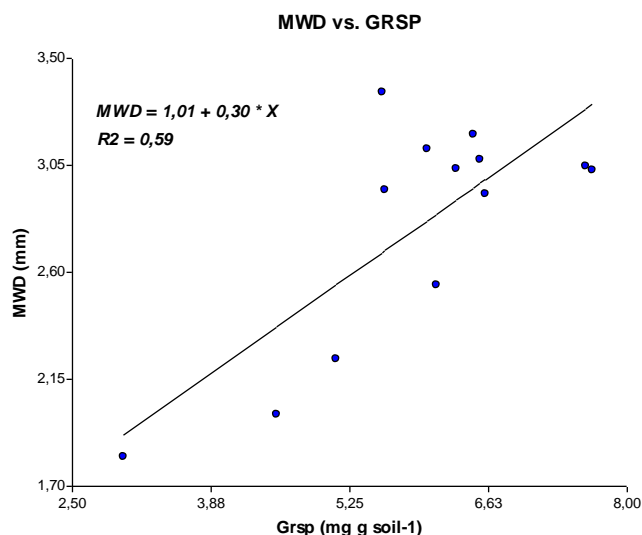
Soils from samples 3, 4 and 7 (grasslands), showed the highest GRSP/Cox ratio: 41, 47 and 57% respectively; these soils present natric conditions (pH greater than 8.2 and elevated exchangeable sodium contents), corresponding to halomorphic soils. This increase in the GRSP/Cox ratio could be linked to the differential expression of glomalin by AMF due to such conditions; Hammer and Rillig (2011) observed that in vitro conditions, the glomalin concentration in arbuscular mycorrhiza hyphae increased with additions of sodium chloride. The other soils, within the range of Cox explored (0.7% to 3.1%), presented values of GRSP/Cox in the range 20% to 39% (Figure 2), which agree with previously reported values (Ferrero Holtz et al., 2016, 2018)



**Figure 2.** GRSP/Cox as a function of Cox (mg g soil-1). The points represent all the test data.

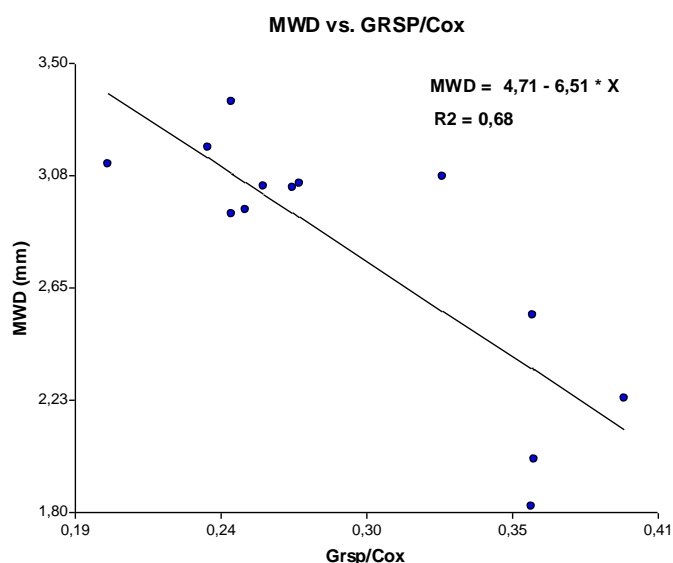
The mean weight diameter of the soil aggregates (MWD) was related to the GRSP contents ( $p < 0.05$ ). A positive linear correlation was observed between MWD and GRSP ( $R^2 = 0.59$ ) (Figure 3).

At humid forests in southern Cameroon, Fokom et al. (2012) analyzed the GRSP and organic matter variables, in order to correlate them with the stability of soil aggregates: aggregate stability showed a positive relationship with both, soil organic matter and GRSP levels, with similar degrees of adjustment ( $R^2 = 0.75-0.8$ ). Wang et al. (2015) observed that increases in exogenous GRSP correlated with the stability of soil aggregates and proposed the hypothesis that GRSP could be considered an agent for the formation and stabilization of soil aggregates. Yang et al. (2017) determined that the MWD were related positively with the contents of GRSP, organic matter and soil organic carbon, which demonstrates the important contributions of these organic components in general to link soil particles in large macro-aggregates and improve their stability.



**Figure 3.** MWD (mm) as a function of GRSP ( $\text{mg g soil}^{-1}$ ). The points do not include soils 3, 4 and 7 because they present massive structure.

Yang et al. (2017), Wang et al. (2015) and Fokom et al. (2012) reported the existence of positive correlation between MWD and GRSP. However, the MWD was negatively related to the GRSP / Cox relationship ( $p < 0.05$ ;  $R^2 = 0.68$ ) (figure 4). These results may be due to the fact that in the more degraded soils, with lower carbon contents, lower absolute GRSP contents were also observed.



**Figure 4.** MWD (mm) as a function of GRSP/Cox. The points do not include soils 3, 4 and 7 because they present massive structure.

#### IV. Conclusions

The content of GRSP was positively correlated with the content of Cox ( $R^2= 0.60$ ). This result indicates that GRSP could be considered a biological indicator of soil, with the accumulation of soil carbon simultaneously with the enrichment of GRSP. However, the GRSP / Cox ratio decreases as Cox increases ( $R^2=0.71$ ), increasing the recalcitrant carbon proportions (GRSP) as the soil carbon decreases. The MWD of soil aggregates correlated positively with the GRSP content ( $R^2= 0.59$ ), although lower values of MWD were associated with higher values of the GRSP / Cox ratio ( $R^2= 0.68$ ).

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