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# Changes in soil aggregation and glomalin-related soil protein content as affected by the arbuscular mycorrhizal fungal species *Glomus mosseae* and *Glomus intraradices*

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## ABSTRACT

Arbuscular mycorrhizal (AM) fungi are key organisms of the soil/plant system, influencing soil fertility and plant nutrition, and contributing to soil aggregation and soil structure stability by the combined action of extraradical hyphae and of an insoluble, hydrophobic proteinaceous substance named glomalin-related soil protein (GRSP). Since the GRSP extraction procedures have recently revealed problems related to co-extracting substances, the relationship between GRSP and AM fungi still remains to be verified. In this work the hypothesis that GRSP concentration is positively correlated with the occurrence of AM fungi was tested by using *Medicago sativa* plants inoculated with different isolates of *Glomus mosseae* and *Glomus intraradices* in a microcosm experiment. Our results show that (i) mycorrhizal establishment produced an increase in GRSP concentration – compared to initial values – in contrast with non-mycorrhizal plants, which did not produce any change; (ii) aggregate stability, evaluated as mean weight diameter (MWD) of macroaggregates of 1–2 mm diameter, was significantly higher in mycorrhizal soils compared to non-mycorrhizal soil, (iii) GRSP concentration and soil aggregate stability were positively correlated with mycorrhizal root volume and weakly correlated with total root volume; (iv) MWD values of soil aggregates were positively correlated with values of total hyphal length and hyphal density of the AM fungi utilized.

The different ability of AM fungal isolates to affect GRSP concentration and to form extensive and dense mycelial networks, which may directly affect soil aggregates stability by hyphal enmeshment of soil particles, suggests the possibility of selecting the most efficient isolates to be utilized for soil quality improvement and land restoration programs.

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## 1. Introduction

Arbuscular mycorrhizal (AM) fungi are mutualistic symbionts living in association with the roots of the majority of land plants. They are key organisms of the soil/plant system, influencing soil fertility and plant nutrition (Smith and Read, 2008). The large network of fungal hyphae, 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). Many

reports have shown that AM fungi are able to counteract soil erosion by increasing the stability of soil aggregates (Andrade et al., 1998; Bethlenfalvay et al., 1999; Miller and Jastrow, 2000) through the combined action of extraradical hyphae and their exudates and residues (Gupta and Germida, 1988; Tisdall and Oades, 1982; Miller and Jastrow, 1990, 1992). Among these fungal components, glomalin, an insoluble and hydrophobic proteinaceous substance (Wright et al., 1996), is of particular interest. Glomalin has been proposed to improve the stability of soil by avoiding disaggregation by water (Wright and Upadhyaya, 1998; Zhu and Miller, 2003; Wright et al., 2007). A strong relationship between glomalin concentration and the amount of water stable aggregates (WSA) has been demonstrated (Wright and Upadhyaya, 1998; Rillig et al., 2001; Harner et al., 2004; Rillig, 2004). Moreover, previous research

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showed that the proportion of WSA higher than 1–2 mm size class is a highly sensitive indicator of the effects of different cropping systems and management practices on soil structure stability (Topp et al., 1997; Marquez et al., 2004).

Glomalin has been detected and quantified in different soil free cultivation systems (Rillig and Steinberg, 2002; Driver et al., 2005; Gadkar and Rillig, 2006), by using a specific monoclonal antibody in ELISA or by Bradford assay. The latter method is also utilized to evaluate glomalin in soil, where it accumulates because of its low turnover rate (Steinberg and Rillig, 2003). However, it has been proposed that the product of soil extraction (121 °C in citrate buffer) evaluated by the Bradford assay represents a proteinaceous material, named glomalin-related soil protein (GRSP), rather than glomalin (Rillig, 2004), since such assay may detect also proteins originated from sources other than AM fungi (Rosier et al., 2006). In addition, recent reports (Jonathan and Javier, 2006; Schindler et al., 2007; Whiffen et al., 2007) have shown that polyphenolic compounds, such as soil tannins and humic acids, may be co-extracted with glomalin and interfere with the Bradford quantification. Thus, in previous field studies on the effect of mycorrhizal symbiosis on soil glomalin concentration (Wright and Upadhyaya, 1998; Rillig et al., 2001; Lutgen et al., 2003), erratic organic matter inputs may have affected data on AM fungal contribution to soil glomalin concentration. As a consequence, although glomalin is assumed to be produced by AM fungi, the relationship between GRSP and AM fungal occurrence remains to be verified.

With the aim of investigating such relationship, and considering that glomalin is probably released in soil by AM fungal extraradical mycelium (Driver et al., 2005), we carried out microcosm experiments with four AM fungal isolates, which had been previously characterized for their ability to develop extraradical mycelium (ERM) showing differences in the extent, structure and interconnectedness of mycelial networks (Avio et al., 2006).

Here we assessed (i) the relationship between mycorrhizal establishment in *Medicago sativa* plants, inoculated with different species and isolates of AM fungi, and GRSP concentration, (ii) the effects of different AM fungi on soil quality variables, such as soil aggregate stability and soil organic matter (SOM), (iii) the relationship between ERM variables of AM fungal isolates and aggregate stability and GRSP concentration.

## 2. Materials and methods

### 2.1. Plant and fungal material

AM fungi used were: *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, isolate IMA1 from UK (collector B. Mosse) and isolate AZ225C from USA (collector J. C. Stutz), and *Glomus intraradices* Schenck & Smith, isolate IMA5 from Italy (collector M. Giovannetti) and isolate IMA6 from France (collector V. Gianinazzi-Pearson). They were obtained from pot cultures maintained in the collection of the Soil Microbiology Laboratory of the Department of Crop Plant Biology, University of Pisa, Italy. The plant species used was the forage legume *M. sativa* cv. Messe.

### 2.2. Experimental set up

Seeds (20) of *M. sativa* were sown into 600 ml plastic pots containing a mixture (1:1, by volume) of soil and Terragreen (calcinated clay, OILDRI, Chicago, IL; pH: 5.1; extractable P: 3.3 ppm). The soil was a sandy loam (clay 15.3%, silt 30.1%, sand 54.6%), with the following characteristics: electrical conductivity ( $\mu\text{S cm}^{-1}$ ), 395.5; organic matter (%), 2.24; total N (%), 1.27; total P (ppm), 469.5; extractable P (ppm), 17.6; extractable K ( $\text{mg kg}^{-1}$ ), 149.6. The mixture was steam-sterilized (121 °C for 25 min, on two

consecutive days), to kill naturally occurring AM fungal endophytes, and pH( $\text{H}_2\text{O}$ ) measured (7.8). Pots were inoculated either with 90 ml of crude inoculum (mycorrhizal roots and soil containing spores and extraradical mycelium) of one of the four fungal isolates or with 90 ml of a sterilized mixture of them (non-mycorrhizal control). Possible differences in AM fungal colonization ability of the four isolates were balanced by using such high amount of inoculum (Lioi and Giovannetti, 1987). All the pots received 120 ml of a filtrate, obtained by sieving a mixture of the four inocula and of agricultural soil from a *M. sativa* field, through a 50  $\mu\text{m}$  diameter pore sieve, to ensure a common microflora to all treatments (Koide and Li, 1989). After emergence, seeds of *M. sativa* were thinned to 10. Plants were grown in greenhouse, supplied with tap water as needed and with a weekly fertilization of half-strength Hoagland's solution (10 ml per pot).

The experiment was a completely randomized design with 5 inoculum treatments (4 fungal isolates and control), and 5 replicates. After four months' growth, plants were harvested, root systems were removed from the pots and stored at  $-20$  °C. Soil was air dried, sieved through a 2 mm diameter pore sieve and then stored at room temperature until processed.

### 2.3. Measurements

The root systems were thawed and dry weights were calculated on subsamples. Three root subsamples from each pot were weighed (ca. 1 g each), thin and thick roots separated and root volumes measured by using an Image Analyzer (Leica Quantimet 500, Milano, Italy). Mycorrhizal colonization was assessed by clearing and staining with Trypan blue in lactic acid (0.05%) and percentages of AM colonization were estimated under a dissecting microscope with the gridline intersect method (Giovannetti and Mosse, 1980). Colonized root volumes were evaluated as total root volume  $\times$  percentage of AM colonization.

GRSP was extracted from soil samples using the procedures described by Wright and Upadhyaya (1996) for easily extractable glomalin (EEG) and total glomalin (TG). Briefly, 1 g of 2 mm sieved soil was placed into a centrifuge tube with 8 ml of a citrate solution, autoclaved and centrifuged at 5000 g for 20 min to pellet the soil particles. The supernatant was decanted and stored at 4 °C until analyzed. Easily extractable glomalin-related soil protein (EE-GRSP) was extracted with 8 ml of a 20 mM citrate solution, pH 7.0, by autoclaving at 121 °C for 30 min. Total glomalin-related soil protein (T-GRSP) was extracted by repeated cycles with 50 mM citrate, pH 8.0, by autoclaving at 121 °C for 60 min. Extractions of samples continued until the supernatant content of glomalin was under method detection limits (ca. 2  $\mu\text{g ml}^{-1}$ ). Extracts from each cycle were pooled and centrifuged at 10,000 g for 10 min to remove residual soil particles. Protein content was determined by Bradford assay (Sigma-Aldrich, Inc.) with bovine serum albumin as the standard. GRSP analyses were carried out on soil from five replicate pots and on three subsamples of the soil mixture obtained before starting the experiment.

Soil structure stability was evaluated by mean weight diameter (MWD) on macroaggregates of 1–2 mm diameter as this size class is considered to be sensitive to short-term treatments of soil (Kemper and Rosenau, 1986). Soil aggregates were obtained by sieving air-dried pot soil stored at room temperature for 4 months. Dried soil samples were carefully broken down by hand into smaller aggregates. The samples, passed through 2.0 mm mesh and retained at 1.0 mm mesh, were used for the following analyses. Water-stability of aggregates was measured using the apparatus and procedure described in Kemper and Rosenau (1986). MWD of water stable aggregates was determined according to van Bavel (1950) and carried out on three subsamples for each replicate. The MWD was

determined on 25 g of soil dry aggregates of less than  $-1.5$  MPa water potential. The samples were overnight wetted by capillary rise, then transferred on the top of a nest of sieves of 1.0, 0.5, 0.25 mm immersed in water. The nest of sieves was then vertically oscillated in water by a shaking machine with a stroke of 4 cm per 5 min, at a rate of 30 complete oscillations per minute. The mass of oven-dried particles ( $105$  °C for 24 h) that resisted breakdown was assessed for each sieve. The mass of the fraction passing through the 0.25 mm sieve was thus obtained by difference. The respective dry masses were used to compute the MWD according to van Bavel (1950), as follows:  $MWD = \sum_{i=1}^n W(i)X_i$ . Where: MWD is the mean weight diameter (mm);  $X_i$  is the arithmetic mean diameter of the  $i$  and  $i - 1$  sieve openings (mm);  $W(i)$  is the proportion of the total sample mass (corrected for sand and gravel) occurring in the  $i$  size fraction (dimensionless);  $n$  is the number of size fractions ( $n = 4$ ).

Soil Organic Matter (SOM) was determined on each replicate using the Walkley–Black wet oxidation method (Nelson and Sommers, 1982).

#### 2.4. Data analysis

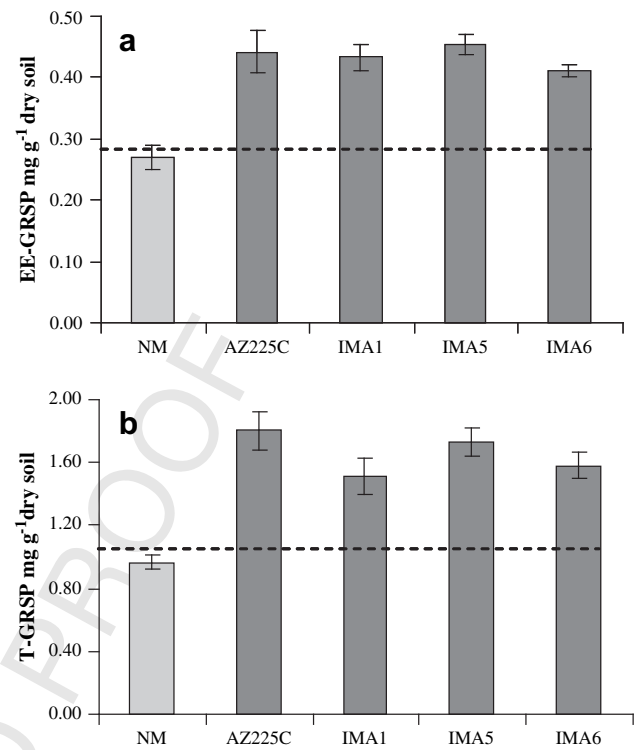
Analysis of variance (ANOVA) was performed on SPSS 16.0 software (SPSS Inc., Chicago, IL), after the necessary transformations and differences between means were assessed by orthogonal contrasts. Analysis of covariance (ANCOVA) was used to separate AM fungal from plant effects, using total root volume as covariate. Pearson correlation coefficients were calculated across plant and soil variables by SPSS software. Data on ERM extent and structure of the different AM fungal isolates of *G. intraradices* and *G. mosseae* utilized in this work were obtained by Avio et al. (2006) using a soilless two-dimensional experimental system, where mycorrhizal plant roots were sandwiched between two cellulose nitrate membranes, buried in sterile quartz grit and maintained in growth chambers. In such system, AM fungal isolates were characterized by differences in extent and interconnectedness of ERM and the phenotypic fungal variables were correlated with plant growth response variables obtained in a microcosm experiment. Here, we utilized the same phenotypic fungal data as explanatory variables, in linear regression analyses, with soil aggregate stability and GRSP concentration as response variables.

### 3. Results

#### 3.1. Soil variables

Soil GRSP contents of mycorrhizal pots, measured as EE-GRSP and T-GRSP, showed large increases compared with the original soil mixture ( $0.29 \pm 0.04$  and  $1.08 \pm 0.04$  mg g<sup>-1</sup> dry soil respectively) (Fig. 1). After four months' growth, mean increases of EE-GRSP and T-GRSP in mycorrhizal pots were 32.4 and 34.9%, respectively, compared to soil mixture values (EE-GRSP,  $P < 0.001$ ; T-GRSP,  $P < 0.001$ ). On the contrary, GRSP contents, both EE-GRSP and T-GRSP, of non-mycorrhizal pots did not show significant differences compared to soil mixture values (EE-GRSP,  $P = 0.28$ ; T-GRSP,  $P = 0.11$ ) (Fig. 1). EE-GRSP and T-GRSP were significantly affected by the mycorrhizal symbiosis (Table 1). Although all the AM fungal strains tested produced similar increases of EE-GRSP, T-GRSP values were significantly different between the two isolates of *G. mosseae* (Table 1; Fig. 1).

Aggregate stability, evaluated as MWD, was significantly higher in mycorrhizal pot soil compared to non-mycorrhizal soil ( $P < 0.001$ ). Soil inoculated with the different AM fungal isolates showed MWD values of 0.68, 0.76, 0.75, 0.90 mm for *G. mosseae* AZ225C, *G. mosseae* IMA1, *G. intraradices* IMA5 and *G. intraradices*



**Fig. 1.** Soil concentration of glomalin-related soil protein (GRSP) in *Medicago sativa* pots inoculated with four isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), or not inoculated (NM). (a) GRSP extracted as easily extractable GRSP (EE-GRSP), (b) GRSP extracted as total GRSP (T-GRSP). Horizontal lines represent soil mixture GRSP values. Bars represent standard errors of the means ( $n = 5$ ).

IMA6, respectively. By contrast, in control pots MWD of soil aggregates was 0.42 mm. Moreover, interspecific differences in MWD values were detected and *G. intraradices* showed also differences between isolates (Table 1).

SOM of mycorrhizal pots was significantly higher compared to controls ( $P < 0.001$ ). SOM values were 1.46, 1.47, 1.66, 1.33% in *G. mosseae* AZ225C, *G. mosseae* IMA1, *G. intraradices* IMA5 and *G. intraradices* IMA6 pots, respectively, and was 0.91% in control soil. Significant differences were observed between *G. intraradices* isolates ( $P = 0.011$ ).

#### 3.2. Plant-fungal variables

Plant root volumes were significantly affected by mycorrhizal symbiosis (Table 2). Root volume increases, calculated for each fungal isolate as  $((\text{root volume mycorrhizal plant} - \text{root volume non-mycorrhizal plant}) / \text{root volume non-mycorrhizal plant}) \times 100$ ,

**Table 1**

*P*-values of linear orthogonal contrasts for easily extractable glomalin-related soil protein (EE-GRSP), total glomalin-related soil protein (T-GRSP) and soil aggregate stability as medium weight diameter of aggregates of 1–2 mm (MWD), of *Medicago sativa* pots inoculated with four isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), or not inoculated (NM).

	EE-GRSP	T-GRSP	MWD
NM vs M	<0.001	<0.001	<0.001
<i>G. mosseae</i> vs <i>G. intraradices</i>	0.806	0.996	0.026
IMA1 vs AZ225C	0.770	0.039	0.197
IMA5 vs IMA6	0.204	0.285	0.024



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**Table 2**  
Root volume and colonized root volume (cm<sup>3</sup> per pot) of *Medicago sativa* plants inoculated with four isolates of the arbuscular mycorrhizal fungal species *Glomus mosseae* (AZ225C and IMA1) and *Glomus intraradices* (IMA5 and IMA6), or not inoculated (NM).

	Root Volume	Colonized Root Volume
NM	32.81 ± 1.65	0.00 ± 0.00
AZ225C	56.23 ± 2.89	34.22 ± 1.28
IMA1	61.62 ± 8.15	34.03 ± 5.73
IMA5	47.56 ± 1.74	32.98 ± 2.83
IMA6	42.16 ± 3.24	33.58 ± 2.61
Treatments compared ( <i>P</i> -values of linear orthogonal contrasts)		
NM vs M	<0.001	<0.001
<i>G. mosseae</i> vs <i>G. intraradices</i>	0.004	0.790
IMA1 vs AZ225C	0.382	0.965
IMA5 vs IMA6	0.382	0.893

Values are means of five replicates pots of each treatment.

were 41.7, 46.8, 31.0 and 22.2% for *G. mosseae* AZ225C, *G. mosseae* IMA1, *G. intraradices* IMA5, *G. intraradices* IMA6, respectively. Although total root volumes did not vary among isolates, highly significant differences were observed at the species level between *G. mosseae* and *G. intraradices* ( $P = 0.004$ ). Plants inoculated with *G. mosseae* showed significantly larger root volumes than those colonized by *G. intraradices* (Table 2).

Colonized root volumes did not vary among AM fungal isolates and showed values ranging from 32.98 to 34.22 cm<sup>3</sup> for *G. intraradices* IMA5 and *G. mosseae* AZ225C, respectively (Table 2).

### 3.3. Relationships between plant, fungal and soil variables

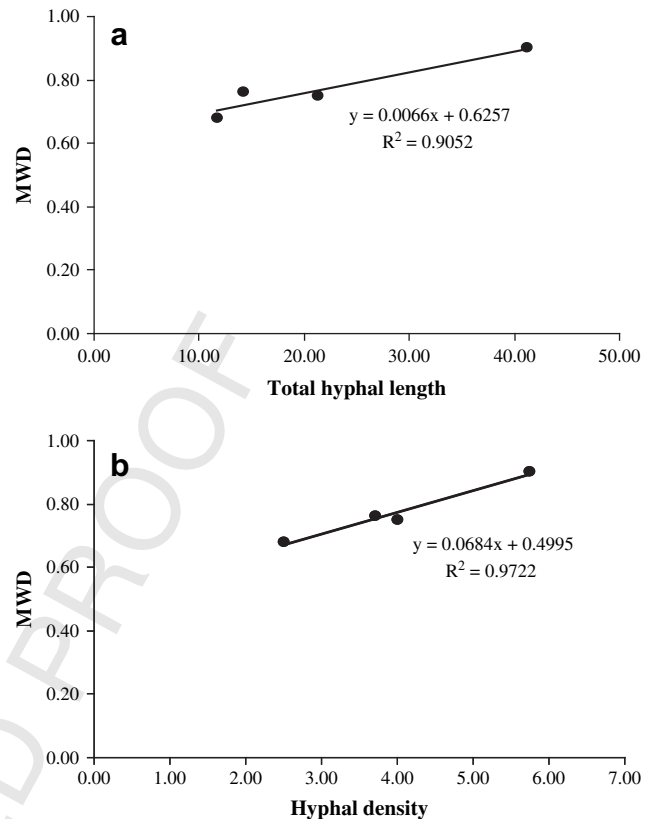
Plant and fungal variables, such as total root volume and colonized root volume, were analyzed for their correlation with soil variables, such as T-GRSP, EE-GRSP and MWD. T-GRSP and EE-GRSP values were more positively correlated with colonized root volume ( $r = 0.696$ ,  $P < 0.001$  and  $r = 0.773$ ,  $P < 0.001$ ), than with total root volume ( $r = 0.453$ ,  $P = 0.023$  and  $r = 0.602$ ,  $P = 0.001$ ). Also MWD values were highly correlated with colonized root volume ( $r = 0.816$ ,  $P = 0.001$ ), but not with total root volume ( $r = 0.315$ ,  $P = 0.295$ ). Soil variables, such as T-GRSP, EE-GRSP and SOM were analyzed for the correlation with soil aggregate stability. MWD was positively correlated with T-GRSP ( $r = 0.759$ ,  $P = 0.003$ ) and EE-GRSP ( $r = 0.653$ ,  $P = 0.016$ ), while was weakly correlated with SOM ( $r = 0.559$ ,  $P = 0.047$ ).

ERM data related to AM fungi, such as total hyphal length and hyphal density, did not show a significant positive relationship with both EE-GRSP and T-GRSP ( $R^2 = 0.55$ ,  $P = 0.260$ ;  $R^2 = 0.11$ ,  $P = 0.660$ ). On the contrary, MWD values of soil aggregates showed a strong relationship with total hyphal length and hyphal density of the corresponding AM fungal isolates ( $R^2 = 0.90$ ,  $P = 0.048$ ;  $R^2 = 0.97$ ,  $P = 0.015$ , respectively) (Fig. 2).

## 4. Discussion

### 4.1. GRSP production by mycorrhizal and non-mycorrhizal plants

Mycorrhizal treatments produced an increase in the initial value of GRSP concentration in pot soil, compared with non-mycorrhizal pots, suggesting a cause-effect relationship between mycorrhizal symbiosis and GRSP content. Our data are direct evidences of differences in GRSP production by mycorrhizal and non-mycorrhizal plants. Many circumstantial evidences, reviewed by Rillig (2004), showed the active role of AM fungi in GRSP production. Positive links between GRSP and AM fungal biomass



**Fig. 2.** Relationships between soil aggregates medium weight diameter (MWD) and total hyphal length (a) and hyphal density (b) for different isolates of *Glomus mosseae* (IMA1 and AZ225C) and *Glomus intraradices* (IMA6 and IMA5). Slopes are significant [ $P = 0.048$  (a),  $0.014$  (b)].

were reported by Rillig et al. (2002), who showed a strong path between the glomalin fraction detected by a specific antibody (IREE-GRSP) and extraradical AM fungal hyphae and by Bedini et al. (2007) who found a good correlation between EE-GRSP and AM fungal spore biovolumes in three differently managed soils. Interestingly, present data are consistent with those obtained by Hallett et al. (2009) who reported difference in GRSP production in mycorrhizal and non-mycorrhizal tomato plants using mutants defective to AM fungal colonization and the AM fungal monoclonal antibody (MAB 32B11). In this work GRSP was assessed using Bradford assay, since Bradford detected protein concentrations are consistent with those detected by indirect enzyme-linked immunosorbent assay (ELISA) (Harner et al., 2004; Nichols and Wright, 2005; Rillig et al., 2006). Here, we evidenced the direct link between Bradford GRSP and AM symbiosis. The large increase in GRSP concentration reported in the present work could be explained also by a contribution of the mycorrhizal plant root system. Actually, we found a higher correlation between GRSP and colonized root volume than between GRSP and total root volume. Since differences in biomass may cause differences in GRSP, we used root volume as a covariate in ANCOVA, to compensate for differences in size between mycorrhizal and non-mycorrhizal plants. When total root volume was used as covariate to separate the effect of mycorrhizal symbiosis from that of plant size on GRSP content, its effects on both EEG-GRSP and T-GRSP were not significant ( $P = 0.605$ ;  $P = 0.805$  respectively). The same results were obtained when using shoot and root plant biomass as covariates on EEG ( $P = 0.312$ ;  $P = 0.268$ , respectively) and on T-GRSP ( $P = 0.117$ ;  $P = 0.684$ , respectively).

#### 4.2. AMF and soil aggregate stability

The presence of AM fungi improved the stability of soil aggregates of the size class of 1–2 mm. MWD values were significantly lower in non-mycorrhizal than in mycorrhizal soils. Interestingly, aggregate stability was more highly correlated with the fraction of organic matter of putative AM fungal origin (TG-GRSP:  $P < 0.001$  and EEG-GRSP:  $P < 0.001$ ), than with total SOM, which has been reported to affect WSA (Tisdall and Oades, 1982; Chan et al., 2002). In addition, as observed for GRSP, aggregate stability was more correlated with colonized root volume than with total root volume, confirming the key role of AM fungi in soil stabilization (Tisdall and Oades, 1982; Miller and Jastrow, 1990). Our results showed that, under microcosm conditions, the stability of soil aggregates is probably affected more by the direct and indirect actions of the plant–fungal system, rather than by plant root metabolism, in agreement with long term field studies, reporting a positive correlation between GRSP and WSA (Wright and Upadhyaya, 1998; Wright and Anderson, 2000; Rillig et al., 2001; Rillig, 2004), and between colonized root length and aggregate stability (Tisdall and Oades, 1982; Miller and Jastrow, 1990, 1992).

#### 4.3. Extraradical mycelium, GRSP and soil aggregation

Interestingly, MWD values of soil aggregates showed a strong relationship with values of total hyphal length and hyphal density of AM fungi, obtained earlier by Avio et al. (2006). Such results suggest that the ability of different AM fungal isolates to form extensive and dense mycelial networks may directly affect soil stability, by hyphal enmeshment of soil particles (Miller and Jastrow, 2000).

By contrast, regression analysis showed no correlation between GRSP and ERM extent, in agreement with previous reports (Rillig et al., 2001; Rillig and Steinberg, 2002; Rillig et al., 2002; Lutgen et al., 2003; Rillig et al., 2003; Lovelock et al., 2004), demonstrating that GRSP cannot be considered as a proxy of ERM fungal biomass and suggesting possible alternative roles for GRSP, as recently reported (Purin and Rillig, 2007).

Here, we showed significant differences among fungal isolates in their ability to affect both GRSP concentration and soil aggregate stability. Such results are in agreement with Wright et al. (1996) who observed differences in the production of glomalin by different *Glomus* species, when extracted directly from hyphae grown in a soilless system.

Our results confirm a previous study concerning the effects of different AM fungal taxa on soil aggregate stabilization (Schreiner et al., 1997). These authors showed that *G. mosseae* improved the stabilization of aggregates of the 2–4 mm size class, significantly more than *Glomus etunicatum* and *Gigaspora rosea*. Though, in contrast with our result, they did not find differences in the size class of 1–2 mm. Moreover, we showed that different isolates of the same AM fungal species (*G. intraradices*) may differ in their capacity of increasing aggregate stability.

AM fungal-mediated contribution as GRSP in soil can be of particular importance in natural and managed ecosystems. Although experimental results obtained under controlled conditions cannot be representative of field conditions, the microcosm approach may help us better understand cause–effect relationships between mycorrhizal symbiosis and soil quality. Our findings highlight the link between GRSP and AM fungi, adding new evidences on their ability to enhance soil aggregate stability. Moreover, the observed differences between AM fungal species evidenced that they may differ not only in morphological and functional characters, but also in ecological traits, such as their ability to stabilize soil aggregates, suggesting the possibility of

selecting the most efficient AM fungal isolates to be utilized for soil quality improvement and land restoration programs.

## 5. Conclusion

The results of our work show that: (i) mycorrhizal establishment in *M. sativa* plants inoculated with different species and isolates of AM fungi produced an increase in GRSP concentration – compared to initial values – in contrast with non-mycorrhizal plants, which did not produce any change; (ii) aggregate stability, evaluated as mean weight diameter (MWD) of macroaggregates of 1–2 mm diameter, was significantly higher in mycorrhizal soils compared to non-mycorrhizal soil, (iii) GRSP concentration and soil aggregate stability were positively correlated with mycorrhizal root volume and weakly correlated with total root volume; (iv) MWD values of soil aggregates were positively correlated with values of total hyphal length and hyphal density of the AM fungi utilized; (v) different AM fungal isolates differently affected GRSP concentration and stability of soil aggregates.

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