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Short communication

Microsite differences in fungal hyphal length, glomalin, and soil aggregate stability in semiarid Mediterranean steppes

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Abstract

Glomalin is a recently discovered glypoproteinaceous substance produced by arbuscular mycorrhizal fungi (AMF) that plays an important role in structuring soil. We quantified soil fungal hyphal length and glomalin content at vegetated and open microsites in *Stipa tenacissima* steppes of SE Spain. Soils underneath the canopy of *S. tenacissima* had higher glomalin pools compared to open microsites. We also found significant differences between sites, suggesting the presence of landscape level heterogeneity in glomalin concentration. Soil fungal hyphal length also differed significantly among the sites, but there was no significant effect of microsite. Water-stable aggregates (1–2 mm diameter; $WSA_{1-2\ mm}$), however, while differing among sites, did not vary as a function of microsite. Furthermore, $WSA_{1-2\ mm}$ was negatively correlated with glomalin fractions, as well as soil organic C. Carbonates were likely the major binding agents in these carbonate-rich (average carbonate content: 71%) soils, and not organic C (including glomalin). AMF-mediated stabilization of soil aggregates did not contribute to the formation and maintenance of fertile islands underneath the canopy of *S. tenacissima*.

Keywords: Carbonates; Restoration; Glomalin; Hyphae; Microsite; Semi-arid steppe; *Stipa tenacissima*

Soil erosion is one of the main threats for the sustainability of arid and semiarid areas (Littleboy et al., 1996; Essiet, 2002), and is one of the main factors that promote desertification (Reynolds, 2001). Soil structure has a prevailing role in soil infiltration and biogeochemical processes, and provides resistance against soil losses. One recently discovered factor that may play an important role in structuring soil is glomalin, a glypoprotein produced by hyphae of arbuscular mycorrhizal fungi (AMF). Glomalin concentration is highly correlated with the percentage of water-stable aggregates in a variety of soils (Wright and Upadhyaya, 1998). Glomalin has been measured in only a few natural ecosystems so far (e.g. several grasslands, deciduous and tropical forest, shrubland; Wright and Upadhyaya, 1998; Rillig et al., 1999, 2000, 2001; Bird et al., 2002), and little is known about variability (horizontal and vertical) of soil glomalin pools, especially in systems with clear habitat microsite-heterogeneity (Bird et al., 2002).

Open steppes dominated by alpha grass (*Stipa tenacissima* L.) are a major vegetation type in the semiarid areas of the Mediterranean Basin (Le Houerou, 2001). In these areas, soils under the canopy of *S. tenacissima* have more organic matter (Puigdefábregas et al., 1999; Maestre et al., 2001), lower temperature (Maestre et al., 2001), higher infiltration capacity (Maestre et al., 2002a), a larger pore volume, higher water storage capacity and greater saturated hydraulic conductivity (Puigdefábregas et al., 1999) than adjacent bare ground areas. These soil changes, in addition to microclimatic amelioration, lead to the facilitation of vascular plants (Maestre et al., 2001, 2002b). Here we test the role of AMF and glomalin in the above-mentioned soil changes induced by *S. tenacissima*. We quantified soil fungal hyphal length, glomalin content and aggregate water stability at vegetated and open microsites in semiarid steppes of SE Spain.

This study was conducted at three *S. tenacissima* steppes located at the province of Alicante, in southeastern Spain (Aguas, 38° 31′N 0° 21′W, 450 m a.s.l., 12° slope, 160° SE aspect; Campello, 38° 30′N 0° 23′W, 380 m a.s.l., 18° slope, 140° SE aspect; Ballestera, 38° 28′N 0° 22′W, 140 m a.s.l., 21°

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Table 1
Effects of site and microsite on glomalin pools and hyphal lengths

	Site	Microsite	Site × microsite
EEG[a]	<0.0001	0.01	0.13
TG	<0.0001	0.01	0.57
IREEG	0.01	0.11	0.09
IRTG	0.02	0.03	0.57
Hyphal length	0.0005	0.14	0.31
CS-$WSA_{1-2\ mm}$	<0.0001	0.18	0.05
CA-$WSA_{1-2\ mm}$	<0.0001	0.28	0.15

P values from 2 × 2 factorial ANOVA. EEG: easily extractable glomalin, TG: total glomalin, IREEG: immunoreactive easily extractable glomalin, and IRTG: immunoreactive total glomalin fraction. Hyphal length is total fungal hyphal length. $WSA_{1-2\ mm}$ = water-sable aggregates (1–2 mm size), CS: carbonates treated as sand, CA: carbonates treated as stable aggregates.
[a] Log transformed.

slope, 170° S aspect). Climate is Mediterranean semi-arid, with a 30 year average annual precipitation ranging from 358 to 388 mm in the studied sites (Pérez Cueva, 1994). Bare ground areas cover between 40 and 55% of total surface. Vegetated patches are dominated by *S. tenacissima* and the sprouting grass *Brachypodium retusum* (Pers.) P. Beauv. The soil is Lithic Calciorthid (Soil Survey Staff, 1990), and its texture (0–20 cm depth) is silty, with 20–47% sand, 36–52% silt and 17–28% clay for the soil under the canopy of *S. tenacissima*, and 20–34% sand, 44–55% silt and 19–27% clay for the soil in bare ground areas (Maestre et al., 2001). Carbonate content in these soils range from 45 to 96% (average: 71%), and pH ranged from 8.1 to 8.7 (Maestre et al., 2001). In spring 2001, we collected soil samples from the upslope of *S. tenacissima* canopy (tussock microsite) and from inter-tussock areas with sparse vegetation cover (open microsite). We sampled five soil cores per site and microsite (3 cm × 3 cm × 15 cm); sample sites were randomly selected in a representative 50 m × 50 m zone within each site.

Glomalin extractions from soil and protein quantifications were carried out as described by Wright and Upadhyaya (1998), yielding four pools of glomalin: easily-extractable glomalin (EEG), total glomalin (TG), and immunoreactive protein (using ELISA with MAb32B11) in these, named IREEG and IRTG. Fungal hyphae were extracted from a 4 g soil subsample by an aqueous extraction and membrane filter technique and quantified as described in Rillig et al. (2002). Water-stability of aggregates was measured (after capillary rewetting) with a wet-sieving method (Kemper and Rosenau, 1986). Percentage of water-stable aggregates (CS-$WSA_{1-2\ mm}$) was calculated using the mass of aggregated soil remaining after wet sieving and the total mass of aggregates at the beginning. The initial and final weights of aggregates were corrected for the weight of coarse matter (>0.25 mm); this treats carbonates in the soil as sand. Since the soils were high in carbonates, we also calculated CA-$WSA_{1-2\ mm}$, which treats carbonate concretions as stable aggregates (Kemper and Rosenau, 1986). This was achieved by separating the sand from carbonates by dissolving the latter in HCl (Kemper and Rosenau, 1986). Organic carbon of soils from the tussock and open microsites in the three study sites were available from another study (Maestre et al., 2001).

Glomalin and hyphal length data were analyzed with a 2 × 2 factorial ANOVA with the factors Site and Microsite (both fixed factors). Regression and correlation analyses were performed to test for relationships between variables measured.

We found a strong pattern in glomalin concentrations at two scales, the site and the microsite, as evidenced by the significant Site and Microsite terms in the ANOVA (Tables 1 and 2). Interaction terms were not significant for any of the pools measured. The tussock microsites had higher glomalin pools compared to the open microsites; this pattern was especially pronounced for the TG pool and quite consistent across the different sites (except for the Aguas site EEG and IREEG pools). Soil fungal hyphal length also differed significantly among the sites (Tables 1 and 2), but there was no significant effect of microsite, nor

Table 2
Glomalin pools (mg g^{-1}) and hyphal lengths (mg^{-1}) at different microsites and sites

	Aguas		Campello		Ballestera	
	Tussock	Open	Tussock	Open	Tussock	Open
EEG	0.59 (0.01)	0.59 (0.04)	0.39 (0.05)	0.27 (0.02)	0.42 (0.02)	0.37 (0.02)
TG	1.80 (0.04)	1.71 (0.10)	1.08 (0.10)	0.87 (0.09)	1.68 (0.03)	1.43 (0.08)
IREEG	0.05 (0.01)	0.06 (0.01)	0.05 (0.01)	0.04 (0.01)	0.10 (0.02)	0.06 (0.01)
IRTG	0.15 (0.02)	0.12 (0.03)	0.09 (0.03)	0.06 (0.01)	0.14 (0.01)	0.07 (0.01)
Hyphal length	21.9 (2.5)	16.8 (1.0)	25.2 (2.6)	26.6 (3.6)	17.2 (2.1)	11.7 (1.7)
CS $WSA_{1-2\ mm}$	70.7 (2.4)	78.7 (2.3)	90.8 (0.8)	90.0 (1.9)	89.8 (1.1)	89.2 (2.4)
CA $WSA_{1-2\ mm}$	79.0 (1.7)	83.1 (2.1)	93.4 (0.7)	92.1 (1.3)	93.4 (0.7)	94.2 (0.7)

Standard errors are in brackets ($n = 5$). EEG: easily extractable glomalin, TG: total glomalin, IREEG: immunoreactive easily extractable glomalin, and IRTG: immunoreactive total glomalin fraction. Hyphal length is total fungal hyphal length. $WSA_{1-2\ mm}$ = water-sable aggregates (1–2 mm size), CS: carbonates treated as sand, CA: carbonates treated as stable aggregates.

Table 3
Pearson's product–moment correlation coefficient (r) matrix for the response variables measured in this study ($n = 30$)

	EEG	TG	IREEG	IRTG	Hyphal length	CS-WSA	CA-WSA
EEG	1.00						
TG	**0.84**	**1.00**					
IREEG	0.24	**0.44**	1.00				
IRTG	**0.70**	**0.75**	**0.36**	1.00			
Hyphal length	−0.13	**−0.43**	−0.21	−0.03	1.00		
CS-WSA	**−0.65**	**−0.49**	0.14	−0.22	0.03	1.00	
CA-WSA	**−0.65**	**−0.44**	0.19	−0.23	−0.09	**0.96**	1.00

Values of r significantly different from zero ($P < 0.05$) are made bold. EEG: easily extractable glomalin, TG: total glomalin, IREEG: immunoreactive easily extractable glomalin, and IRTG: immunoreactive total glomalin fraction. Hyphal length is total fungal hyphal length. $WSA_{1-2\ mm}$ = water-sable aggregates (1–2 mm size), CS: carbonates treated as sand, CA: carbonates treated as stable aggregates.

was the interaction term significant (Table 1). While glomalin pools were mostly lowest in the Campello site, hyphal lengths were highest at that site. $WSA_{1-2\ mm}$ differed among sites, but not between microsites (Tables 1 and 2). The different glomalin fractions were frequently well correlated with each other (Table 3). Hyphal length was significantly negatively correlated with the TG fraction, but not correlated with other fractions of glomalin or aggregate water-stability (Table 3). The two different WSA calculations (based on different treatment of carbonates as either sand or stable aggregates) were highly positively correlated with each other. Strikingly, WSA was significantly negatively correlated with EEG and TG and not correlated with IREEG and IRTG (Table 3). There was a positive linear correlation of glomalin (IRTG is presented, but pattern was similar for TG) with soil organic C (Fig. 1). Since glomalin (e.g. TG and IRTG) were significantly positively correlated with soil organic C, the latter was also significantly negatively correlated with WSA (data not shown).

Glomalin pools in this tussock system were clearly spatially structured. Our data show the presence of a strong microsite effect on this fungal protein, similar to data collected previously in a North American semi-arid ecosystem (Bird et al., 2002), where higher concentrations of glomalin (and greater aggregate water-stability) were found underneath *Bouteloua eriopoda* [Torr.] Torr and *Prosopis glandulosa* Torrey compared to open interspaces. The differences found between tussock and open microsites in this fungal protein were hypothesized to play an important role in the improvement of soil structure underneath the canopy of *S. tenacissima*, thereby promoting the formation of fertile islands under the canopy of this tussock grass (Puigdefábregas et al., 1999). However, we found no evidence in this study that this occurs. In fact, contrary to all previous reports (e.g. Wright and Upadhyaya, 1998; Bird et al., 2002), glomalin pools were either significantly negatively correlated with WSA, or not at all (Table 3). This suggests that glomalin was not the main aggregate binding agent in these carbonate-rich soils. Aggregates are mainly stabilized by carbonate concretions in these Calcisols (Kemper and Rosenau, 1986). At a landscape scale, there were also strong differences among the different sampling sites. Differences between sites could be related to small-scale differences in rainfall and vegetation cover (Maestre et al., 2001).

Previous studies have shown that *S. tenacissima* is highly colonized (70–80%) by AMF (Requena et al., 1996). As roots of this species do not harvest resources from the open areas and are largely restricted to the soil underneath the canopy (Puigdefábregas et al., 1999), the lack of significant differences in hyphal length between tussock and open microsites was surprising (although there was a trend with $P = 0.14$; Table 1). However, at the study sites the number of mycorrhizal propagules is similar in both tussock and open microsites (Azcón-Aguilar et al., 2003). We did not distinguish AMF hyphae from those of other fungi. Hence, it is unclear whether open microsites had a smaller proportion of AMF hyphae and a higher proportion of saprobic fungal hyphae.

Restoration practices use the nurse effect provided by *S. tenacissima* tussocks to facilitate establishment of native

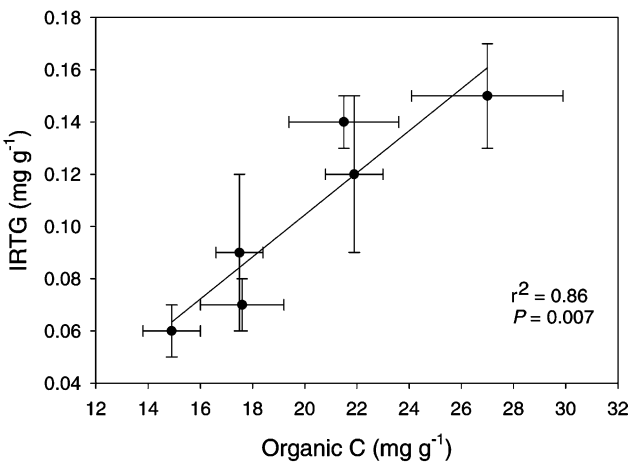

Fig. 1. Linear relationship of immunoreactive total glomalin (IRTG; mg g^{-1} soil) with soil organic carbon (C). Data points represent means ± SE ($n = 5$). C data are from Maestre et al. (2001).

shrubs in degraded steppes (Maestre et al., 2001, 2002b). Our study showed that the higher glomalin concentrations in the tussock microsites did not translate into increased aggregate stability, suggesting that AMF are not directly involved in improving soil structure of the tussock sites. This system provides an opportunity to examine the role of glomalin in soils in the absence of its (typical) effects on soil aggregation.

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