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## The response of glomalin-related soil proteins to fire or tillage

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#### ABSTRACT

Glomalin-related soil proteins (GRSP) are glycoproteins abundantly produced by arbuscular mycorrhizal fungi in roots and soil. They can be a significant component of soil organic matter, which effectively acts to bind mineral particles together, so improving soil structure. Although it is known that high temperatures and tillage both have a negative impact on GRSP, there is no study aimed at comparing and contrasting the effects of fire and enduring cultivation on GRSP content in contiguous areas undergoing the two forms of soil disturbance. We studied such a situation in Kurdistan, Iran, where the top 5 cm of soil was sampled in *i*) an unmanaged and unburned oak sparse forest, both inside and outside sprout clumps, *ii*) a vineyard, both under and outside the canopy, and *iii*) a burned portion of the forest in between. In the latter, the samples were collected from areas burned with high and moderate severity inside sprout clumps, and areas burned with low severity outside sprout clumps. All soil samples were air-dried and sieved to obtain four aggregate size fractions (8–2, 2–1, 1–0.25, and < 0.25 mm in diameter). Total organic carbon (TOC), easily extractable glomalin (EEG) and residual glomalin (RG) were determined in all fractions.

Both TOC and GRSP were significantly lower in the cultivated soil than in the unburned forest. Fire did not change the content of TOC, although GRSP increased with increasing fire severity. The relative amount of small aggregates was higher in the burned and cultivated soils, likely because of the breakdown of macroaggregates by heating and tillage, respectively. TOC, EEG, and RG generally increased in concentration with decreasing aggregate size. The linear regression analysis revealed significant relations between GRSP and TOC in all studied treatments; however, the GRSP/TOC ratio was significantly higher in the soil burned with the highest severity and in the vineyard compared to the unburned soil- Overall, this study demonstrated that GRSP in soil is significantly affected by both severe fire and cultivation, and suggests that total amount of GRSP and GRSP distribution between the aggregate size fractions might be two reliable indicators of these types of perturbations of the soil environment.

#### 1. Introduction

Microbiological attributes are used to account for ongoing variations from the steady state of soils, because the biomass, diversity, and activity of soil organisms change significantly in response to humaninduced or natural perturbations with over time scales much shorter than other soil properties (Anderson, 2003; Nogueira et al., 2006). Wildfires are among the most frequent causes of significant soil biota alteration (Prendergast-Miller et al., 2017), due to both the direct effect of high temperatures and the indirect effect of changes experienced by the vegetation and the soil (Hart et al., 2005; Certini, 2014). Arbuscular mycorrhizal fungi (AMF), which through the production of extracellular compounds and extensive hyphae networks play a crucial role in many soil processes (Peng et al., 2013), are significantly affected by wild or controlled fire (Bellgard et al., 1994; Pattinson et al., 1999). AMF are main producers of glomalin, a component of AMF spores and hyphal wall that is finally released to the soil environment (Driver et al., 2005) and can be a substantial portion of soil organic matter (Lovelock et al., 2004). Glomalin is quantified operationally as glomalin-related soil proteins (GRSP), which are N-linked glycoproteins with brown to dark red-brown colour and comprising 3 to 5% nitrogen, 36 to 59% carbon, 4 to 6% hydrogen, 33 to 49% oxygen, and 0.03 to 0.1% phosphorous (Lovelock et al., 2004; Schindler et al., 2007). GRSP also contain 0.8 to 8.8% Fe (Wright and Upadhyaya, 1998; Rillig et al., 2001), which is the reason for the reddish colour of the glomalin extracts. GRSP are stable in soil, because they are insoluble in water and resistant to heat degradation (Wright et al., 1996). Their concentration in soil has been reported to amount from less than  $1 \text{ mg g}^{-1}$  in a desert in northern China (Bai et al., 2001). With ascertained turnover times of even

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Table 1
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	pН	EC ( $dSm^{-1}$ )	Bd (g cm <sup>-3</sup> )	SMC (%)	ECC (%)	Clay (%)	Silt (%)	Sand (%)
FO	$7.5 \pm 0.1^{cd}$	$0.21 \pm 0.0^{c}$	$1.2 \pm 0.1^{a}$	$6.0 \pm 0.3^{cd}$	$1.6 \pm 0.1^{cd}$	$39.4 \pm 4.1^{a}$	$41.0 \pm 3.7^{b}$	$19.5 \pm 0.4^{\rm b}$
FI	$7.3 \pm 0.2^{d}$	$0.26 \pm 0.0^{bc}$	$0.8 \pm 0.1^{\circ}$	$13.7 \pm 0.7^{a}$	$2.6 \pm 0.2^{b}$	$34.6 \pm 3.8^{ab}$	$41.6 \pm 4.8^{b}$	$23.8 \pm 1.5^{b}$
BLO	$7.6 \pm 0.0^{bcd}$	$0.23 \pm 0.0^{\circ}$	$1.1 \pm 0.1^{ab}$	$6.4 \pm 0.4^{bc}$	$1.5 \pm 0.2^{cd}$	$38.8 \pm 0.3^{a}$	$41.3 \pm 1.0^{b}$	$19.9 \pm 1.1^{b}$
BMI	$7.8 \pm 0.0^{abc}$	$0.29 \pm 0.0^{\rm b}$	$1.0 \pm 0.1^{ab}$	$6.6 \pm 0.3^{bc}$	$2.3 \pm 0.1^{\rm bc}$	$33.6 \pm 1.6^{abc}$	$41.1 \pm 2.1^{b}$	$25.2 \pm 2.5^{ab}$
BHI	$7.9 \pm 0.0^{ab}$	$0.52 \pm 0.0^{a}$	$0.9 \pm 0.0^{\rm bc}$	$8.0 \pm 1.0^{b}$	$5.6 \pm 0.7^{a}$	$25.5 \pm 1.8^{cd}$	$44.4 \pm 0.4^{ab}$	$30.1 \pm 1.6^{a}$
VO	$8.0 \pm 0.2^{a}$	$0.23 \pm 0.0^{\circ}$	$1.1 \pm 0.0^{ab}$	$4.2 \pm 0.7^{d}$	$1.0 \pm 0.1^{d}$	$22.6 \pm 3.5^{d}$	$53.5 \pm 3.2^{ab}$	$23.8 \pm 1.3^{b}$
VI	$7.9 \pm 0.1^{ab}$	$0.23 \pm 0.0^{\circ}$	$1.1 \pm 0.1^{ab}$	$5.5 \pm 0.6^{cd}$	$1.3 \pm 0.1^{d}$	$28.5 \pm 1.5b^{cd}$	$49.4 \pm 3.2^{a}$	$22.2 \pm 3.0^{bc}$

<sup>1</sup> Different superscript letters indicate significant differences (P < 0.05) between means in each column according to Duncan Multiple Range Test. BD: soil bulk density, SMC: soil moisture content, ECC: equivalent calcium carbonate. Unburned forest outside sprout clumps (FO) and inside sprout clumps (FI), forest burned with low severity outside sprout clumps (BLO), forest burned with moderate severity inside sprout clumps (BMI), forest burned with high severity inside sprout clumps (BHI), vineyard outside the canopy (VO) and under the canopy (VI).

#### Table 2

Easily-extractable glomalin (EEG), residual glomalin (RG), and total organic carbon (TOC) contents in aggregate size fractions of soils from the different treatments. Values are means of three replicates  $\pm$  the standard errors<sup>1</sup>.

Treatments	Aggregate size (mm)	EEG (mg $g^{-1}$ )	RG (mg $g^{-1}$ )	TOC (mg $g^{-1}$ )
FO	8-2 2-1	$1.2 \pm 0.1^{a}$ $1.3 \pm 0.1^{a}$	$7.5 \pm 0.9^{a}$ $7.6 \pm 0.7^{a}$	$59.1 \pm 1.1^{b}$ $65.7 \pm 5.4^{ab}$
	1–0.25 < 0.25	$1.5 \pm 0.2^{a}$ $1.6 \pm 0.2^{a}$	$8.3 \pm 0.8^{a}$ $8.8 \pm 0.7^{a}$	$72.2 \pm 2.7^{a}$ $77.1 \pm 4.4^{a}$
FI	< 0.23 8–2	$1.0 \pm 0.2$ $1.4 \pm 0.1^{b}$	$10.2 \pm 0.4^{b}$	$63.4 \pm 1.5^{\circ}$
	2-1	$1.8 \pm 0.3^{ab}$	$10.2 \pm 0.7^{\rm b}$ $11.2 \pm 0.7^{\rm b}$	$79.2 \pm 5.2^{b}$
	1-0.25	$2.1 \pm 0.3^{ab}$	$15.1 \pm 1.3^{a}$	$99.8 \pm 6.0^{a}$
	< 0.25	$2.3 \pm 0.2^{a}$	$16.4 \pm 0.3^{a}$	$112.2 \pm 4.5^{a}$
BLO	8–2	$1.1 \pm 0.1^{b}$	$6.2 \pm 0.1^{c}$	$60.9 \pm 1.4^{a}$
	2–1	$1.1 \pm 0.0^{\mathrm{b}}$	$6.3 \pm 0.2^{c}$	$53.9 \pm 1.4^{b}$
	1-0.25	$1.5 \pm 0.2^{ab}$	$7.1 \pm 0.3^{b}$	$65.0 \pm 1.4^{a}$
	< 0.25	$1.6 \pm 0.1^{a}$	$8.6 \pm 0.3^{a}$	$64.2 \pm 3.2^{a}$
BMI	8–2	$1.4 \pm 0.1^{c}$	$9.7 \pm 0.1^{b}$	$58.0 \pm 3.2^{b}$
	2–1	$1.5 \pm 0.1^{\circ}$	$10.4 \pm 0.7^{b}$	$60.4 \pm 4.5^{b}$
	1-0.25	$2.0 \pm 0.0^{b}$	$15.0 \pm 0.5^{a}$	$76.0 \pm 7.4^{ab}$
	< 0.25	$2.5 \pm 0.1^{a}$	$17.1 \pm 1.5^{a}$	$90.7 \pm 8.1^{a}$
BHI	8–2	$1.9 \pm 0.1^{c}$	$13.4 \pm 0.5^{c}$	$69.6 \pm 6.7^{b}$
	2–1	$2.3 \pm 0.1^{\circ}$	$15.3 \pm 0.4^{c}$	$73.0 \pm 11.0^{b}$
	1-0.25	$2.7 \pm 0.1^{b}$	$17.2 \pm 1.0^{b}$	$91.0 \pm 8.4^{a}$
	< 0.25	$3.0 \pm 0.0^{a}$	$20.8 \pm 0.6^{a}$	$92.8 \pm 1.6^{a}$
VO	8–2	$1.2 \pm 0.1^{a}$	$5.9 \pm 0.3^{a}$	$31.8 \pm 1.9^{ab}$
	2–1	$1.1 \pm 0.1^{a}$	$6.1 \pm 0.1^{a}$	$29.2 \pm 0.8^{b}$
	1-0.25	$1.3 \pm 0.2^{a}$	$6.7 \pm 0.2^{a}$	$32.7 \pm 0.4^{ab}$
	< 0.25	$1.6 \pm 0.2^{a}$	$7.3 \pm 1.2^{a}$	$34.7 \pm 1.0^{a}$
VI	8–2	$1.3 \pm 0.1^{a}$	$7.6 \pm 0.4^{a}$	$30.3 \pm 1.3^{b}$
	2-1	$1.3 \pm 0.1^{a}$	$8.1 \pm 0.6^{a}$	$36.7 \pm 0.2^{a}$
	1-0.25	$1.2 \pm 0.1^{a}$	$8.1 \pm 0.2^{a}$	$39.0 \pm 1.6^{a}$
	< 0.25	$1.6 \pm 0.1^{a}$	$8.2 \pm 0.4^{a}$	$41.3 \pm 1.7^{a}$

<sup>1</sup> Different superscript letters indicate significant differences (P < 0.05) between means in each column according to Duncan Multiple Range Test. Unburned forest outside sprout clumps (FO) and inside sprout clumps (FI), forest burned with low severity outside sprout clumps (BLO), forest burned with moderate severity inside sprout clumps (BMI), forest burned with high severity inside sprout clumps (BHI), vineyard outside the canopy (VO) and under the canopy (VI).

decades, GRSP are part of the stable soil organic matter pool (Rillig et al., 2001). GRSP efficiently bind mineral particles together, so improving soil structure and related properties, such as porosity, hydraulic conductivity, and resistance to capping, crusting, compaction, and erosion (Wright et al., 1996; Singh, 2012); therefore, GRSP preservation should be a purpose of every type of soil management.

Northern Zagros oak forest (hereafter called NZOF), counting 5 million ha, is a major reservoir of carbon and biodiversity of the Kurdistan province, west Iran (Haidari et al., 2013). Overgrazing since long time, extensive conversion to vineyard during last 20–30 years, and frequent uncontrolled wildfires more recently are the human-induced disturbances causing decline of this forest. In fact, all of them are

known to have several negative consequences for soil conservation; cultivation and wildfires especially *via* carbon (C) loss and consequent soil structure degradation (Shepherd et al., 2002; Sharifi et al., 2017) and reduction in amount and diversity of soil organisms (Verma and Jayakumar, 2012), including AMF (Jansa et al., 2003; Brito et al., 2012).

This work aims at checking the effect of a single wildfire and cultivation perpetuated over time on the concentration of soil glomalin (for simplicity, hereafter we will use this specific term for GRSP as well) in different aggregate size fractions and its role on aggregate stability. For this purpose, we compared and contrasted three adjacent areas in the NZOF: the unburned forest, a portion of the forest burned three years earlier, and a vineyard planted thirty years earlier in place of the forest.

#### 2. Materials and methods

#### 2.1. Study area and experimental design

The research was carried out in the Zagros forest, which extends over 1300 km in western Iran. In particular, the study site was in the northern side of the forest, in the Marivan region, Kurdistan Province, close to the village of Nezhmar (46° 6' E to 46° 10' E, 35° 31' N to 35° 35' N). The climate of the area is sub-humid, consisting of cold and rainy winters, and warm and dry summers. The mean annual temperature is 12.8 °C and the mean annual precipitation is 991.2 mm, most of which is confined in January to May. Soils are calcareous, moderately thick to thick, and have textures from silty clay to silty clay loam. The landscape ranges from steep terrain covered with forests and vineyards to flat plains cultivated for wheat, alfalfa, and vegetables. Prevailing are coppices of Persian oak (Quercus brantii Lindl) and Aleppo oak (Quercus infectoria Olivier), consisting in sparse clumps of higher vegetation. The herbaceous plants in the forest belong to the Asteraceae, Poaceae, Fabaceae, Lamiaceae, Brassicaceae, and Apiaceae families (Haidari and Rezaei, 2013). The majority of these plants have ectomycorrhizae and/or endomycorrhizae in their root system, which could imply abundance of GRSP in soils of the area.

An extensive conversion of the forest to vineyards during the last decades, especially following arsons, supplemented the atavistic over grazing, so leading to significant reduction of the original forest. The vineyards are usually ploughed down to 30 cm and pruned every year in early spring. Tillage clearly accelerated soil erosion, with substantial amounts of topsoil shifted from the vineyards to lowlands.

Three areas along an east-west oriented transect lying on a back slope were studied, all showing the same slope (20–25%), elevation (1320 m a.s.l.), and ground morphology. One was the natural oak forest, another one a part of the forest burned three years earlier, and the third one a vineyard planted thirty years earlier in place of the forest. Fire severity in the burned area was varied, chiefly because of the heterogeneity of fuel amount and type. The study areas were close

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