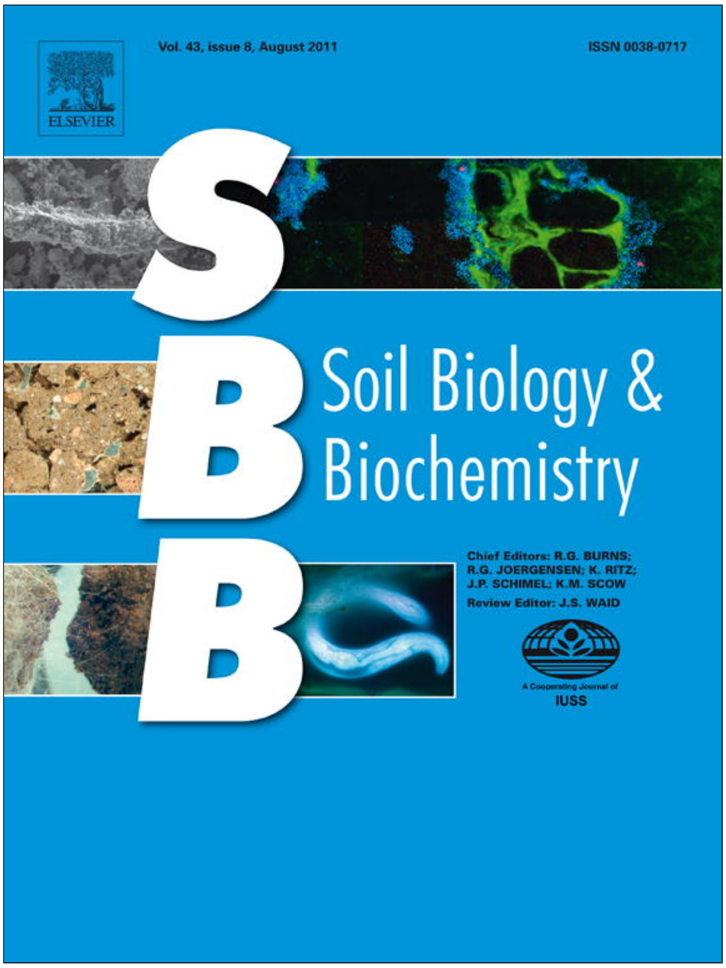
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Short Communication

Shrub encroachment does not reduce the activity of some soil enzymes in Mediterranean semiarid grasslands

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Shrub encroachment is a worldwide phenomenon with implications for desertification and global change. We evaluated its effects on the activities of urease, phosphatase and β-glucosidase in Mediterranean semiarid grasslands dominated by *Stipa tenacissima* by sampling 12 sites with and without resprouting shrubs along a climatic gradient. The presence of shrubs affected the evaluated enzymes at different spatial scales. Soils under *S. tenacissima* tussocks and in bare ground areas devoid of vascular plants had higher values of phosphatase and urease when the shrubs were present. For the β-glucosidase, this effect was site-specific. At the scale of whole plots (30 m × 30 m), shrubs increased soil enzyme activities between 2% (β-glucosidase) and 22% (urease), albeit these differences were significant only in the later case. Our results indicate that shrub encroachment does not reduce the activity of extracellular soil enzymes in *S. tenacissima* grasslands.

Shrub encroachment, i.e. the increase in the density and cover of shrub species in former grasslands, is a phenomenon widely documented in arid and semiarid areas worldwide (Schlesinger et al., 1990; Parizek et al., 2002; Franco and Morgan, 2007; Maestre et al., 2009). This land-use strongly impacts the composition and functioning of ecosystems in manner that may lead to the onset of desertification processes (Schlesinger et al., 1990). The linkages between shrub encroachment and desertification constitute a current paradigm in dryland ecology (e.g. Peters and Havstad, 2006; Okin et al., 2009), and has even reached the management and political arena (e.g. MEA, 2005). However, it has been recently shown that shrub encroachment may not have negative effects on nutrient cycling processes (McKinley et al., 2008), and may even promote the reversal of desertification (Maestre et al., 2009). Many studies have been conducted to evaluate how shrub encroachment affect biogeochemical processes (e.g., Schlesinger et al., 1990; Parizek et al., 2002; Hibbard et al., 2001; McKinley et al., 2008). However, the effects of this phenomenon on extracellular soil enzymes produced by bacteria and fungi, such as urease, phosphatase and β-glucosidase, are virtually unknown. To fill this gap, we evaluated the effects of shrub encroachment on the activities of urease, phosphatase and β-glucosidase in Mediterranean semiarid grasslands dominated by *Stipa tenacissima* L.

Our study was conducted at 12 sites located along a climatic gradient spanning from the center to the south-eastern coast of Spain (Appendix 1). Their average annual precipitation and temperature range from 265 mm to 497 mm and from 13 °C to 17 °C, respectively. Eleven of the sites were located on *Lithic Calciorthid* soils, while another was located on *Typic Gypsiorthid* soils (Soil Survey Staff, 1994). All the sites had south-facing slopes. Vegetation was in all cases an open grassland dominated by *S. tenacissima*, with total plant cover below 70%. At each site, we established two 30 m × 30 m paired plots separated by distances lower than 1000 m. One of the plots (SH plot) was located in areas with well-developed adult individuals of resprouting shrubs (mostly *Quercus coccifera* L. and *Rhamnus lycioides* L.), covering from 4% to 40% of total plant cover (Appendix 1). The other (NSH plot) was established in areas without resprouting shrubs (0.4% of total perennial cover in average, Appendix 1). Paired plots were placed in areas with similar slope, total cover and orientation.

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Soil sampling was conducted between 20 July and 15 August 2006. In the NSH plots, five sampling points were randomly placed in bare ground areas and *S. tenacissima* tussocks ("bare" and "tussock" microsites, respectively). A composite sample obtained from five 145 cm^3 soil cores (0–7.5 cm depth) was obtained for each sampling point, bulked and homogenized in the field. In the SH plots, the same scheme was followed, but additional samples were collected from the soil located under the canopy of five randomly selected shrubs ("shrub" microsite). Soil samples were transported to the laboratory, where they were sieved by 2 mm mesh and air-dried for four weeks. The activities of urease, phosphatase and β-glucosidase were determined according to Nannipieri et al. (1980), Tabatabai and Bremner (1969), and Tabatabai (1982), respectively. These variables were measured using air-dried soil samples. Previous studies have found that the enzymes evaluated are hardly affected by air-drying in semiarid Mediterranean soils (Zornoza et al., 2006, 2009), which otherwise are under dry conditions most of the year (e.g., Maestre et al., 2002).

Data from the tussock and bare microsites were analyzed using a three-way nested ANOVA. It included Site as random between-plot factor, Shrubs (S and NSH plots) as a fixed factor and Microsite (bare and tussock) as a within-plot fixed factor (Quinn and Keough, 2002). We estimated the three soil enzyme activities at the scale of each 30 × 30 m plot by multiplying the proportion of the area covered by the tussock, bare and shrub microsites by the average enzyme activities obtained at each microsite. The effects of shrubs on soil enzyme activities at this spatial scale were evaluated using paired *t*-tests, where values obtained at each site acted as replicates. All the statistical analyses were conducted with the software SPSS for Windows 14.0 (SPSS Inc., Chicago, IL, USA). All the interpretations of the statistical analyses were done by evaluating the raw *P* values (Gotelli and Ellison, 2004).

Higher values of the activities of phosphatase and β-glucosidase were found in the tussock microsite compared to the bare microsite (Fig. 1A and B; Table 1). The magnitude of the differences between tussock and bare microsites was site-specific in the case of phosphatase and urease, as revealed by a significant Site × Microsite interaction (Table 1, Appendix 2). The presence of shrubs increased the content of the phosphatase and urease enzymes in both tussock and bare microsites (Fig. 1A and C), as indicated by a significant Shrub effect (Table 1). This effect was site-specific for the β-glucosidase, as revealed by a significant Shrub(Site) × Microsite interaction (Appendix 2, Table 1). At the plot scale, the activity of the three soil enzymes evaluated was higher in the SH plots (between 2% and 22% increase, Fig. 1), albeit these differences were significant only in the case of urease (urease: $t_{11} = -2.315$, $P = 0.041$; phosphatase: $t_{11} = -1.415$, $P = 0.185$; β-glucosidase: $t_{11} = -0.822$, $P = 0.418$).

The presence of shrubs increased the activity of phosphatase, urease and β-glucosidase in soils from the tussock and bare microsites, suggesting that the effects of shrubs on these enzymes were evident in areas outside their canopies. Soils from the tussock and bare microsites have more nutrients (N, P and K), microbial activity and organic carbon in SH than in NSH plots (Maestre et al., 2009). Furthermore, the activities of phosphatase and β-glucosidase were positively related to variables such as total soil N and P, potential N mineralization and soil respiration in our study sites (Appendix 3). These results suggest that increases in overall soil fertility and organic matter promoted by shrub encroachment are enhancing the activities of extracellular enzymes at the tussock and bare microsites. Alternative explanations, such as historical legacies of high N and P microsites favoring shrub establishment, albeit cannot be fully excluded, are unlikely to explain these results because resprouting shrubs present in *S. tenacissima* grasslands do not need high fertility levels to establish themselves (Caravaca et al., 2005; Trubat et al., 2008), and because the increases in soil fertility promoted by shrubs were quite consistent along the sites studied (Maestre et al., 2009). Attributes of shrub patches that may lead to local increases in overall fertility include enhanced water infiltration and root exudate production (Puigdefábregas et al., 1999; Archer et al., 2002), and higher litter quality (the litter of shrubs like *Q. coccifera* shows C:N values between 40 and 50 [Ferran

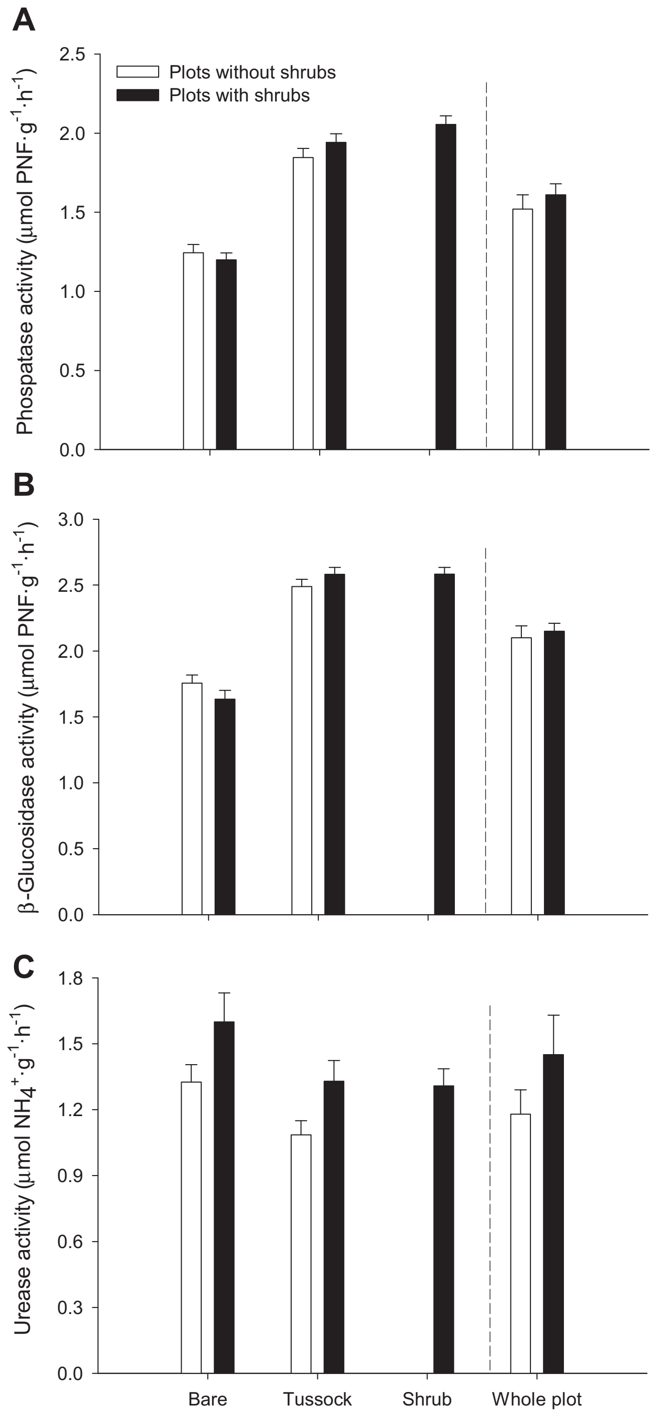

Fig. 1. Values of measured enzymatic activities in plots with and without shrubs. Bare, Tussock and Shrubs indicate soils collected in bare ground areas, under the canopy of *Stipa tenacissima* tussocks and under the canopy of resprouting shrubs, respectively. Whole plot indicates values estimated for 30 m × 30 m plots. Data from all the sites are pooled. Data represent means ± SE ($n = 60$ excepting for whole plot estimates, where $n = 12$).

Table 1
Nested ANOVAS for main treatment effects and interactions on soil enzyme activity data from the tussock and bare microsites. S = Site, SH = Shrub (plots with/without shrubs), and M = microsite. *P* values below 0.05 are shown in boldface.

Source of variation	df	Phosphatase			β-Glucosidase			Urease[a]		
		MS	F	*P*	MS	F	*P*	MS	F	*P*
S	11	1.55	5.12	**0.004**	1.15	3.29	**0.026**	0.32	3.65	**0.017**
SH(S)	12	0.30	5.04	**0.004**	0.35	1.31	0.323	0.09	5.07	**0.004**
M	1	27.10	62.47	**<0.001**	42.32	58.53	**<0.001**	0.26	4.77	0.051
S × M	11	0.43	7.25	**0.001**	0.72	2.71	0.051	0.05	3.21	**0.028**
SH(S) × M	12	0.05	0.89	0.557	0.27	2.26	**0.011**	0.02	0.74	0.713
Residual	192	0.07			0.12			0.02		

[a] Analyses were conducted with $\log_{10}$-transformed data.

et al., 2004], while that of *S. tenacissima* typically varies from 55 to 85 [Maestre and Cortina, 2006]). Other potential mechanism that could help to explain our results is the transfer of C and nutrients between shrub patches and bare ground areas containing biological soil crusts via soil fungi (Green et al., 2008). Neither the number of mycorrhizal propagules nor hyphal length are negligible in bare microsites in *S. tenacissima* grasslands (Azcón-Aguilar et al., 2003; Rillig et al., 2003), which are also dominated by biological soil crusts (Maestre et al., 2009).

When extrapolating to whole plots, SH plots had higher enzyme activities than NSH plots, albeit the differences were significant only in the case of urease. Increases in net primary productivity (NPP) and atmospheric N fixation following shrub encroachment of former grasslands by species such as *Prosopis glandulosa* have often been cited as key drivers underlying biogeochemical changes associated to this phenomenon (Liao and Boutton, 2008). None of the resprouting shrubs found at our study sites is able to fix atmospheric nitrogen, but species like *Q. coccifera* commonly show higher NPP than *S. tenacissima* (365–472 g m^{-2} yr^{-1} vs. 279–411 g m^{-2} yr^{-1}; Cañellas and San Miguel, 1998; Puigdefábregas and Mendizábal, 1998). However, total plant cover did not differ between NS and NSH plots (Appendix 1, paired *t*-test: $t_{11} = -0.506$, $P = 0.623$), and the magnitude of the effect of shrub encroachment on soil enzyme activities did not vary with changes in the abundance of resprouting shrubs (Appendix 4). These results suggest that changes in NPP alone are unlikely to explain the patterns observed. Woody vegetation has positive effects on rabbit (*Oryctolagus cunniculus* L.) abundance during summer in semi-arid Mediterranean grasslands (Rueda et al., 2008). Rabbits are common in all of our study sites (Maestre et al., 2009), and we suggest that the increase in the activity of urease found in SH plots, which cannot be linked to their overall higher soil fertility (Appendix 3), could have been caused by changes in rabbit abundance in these plots.

Shrub encroachment is often considered a form of desertification, with negative consequences for ecosystem structure and functioning (Schlesinger et al., 1990; Peters and Havstad, 2006; Okin et al., 2009). Contrarily to this view, we found that shrub encroachment do not have negative effects on soil enzyme activity in semi-arid *S. tenacissima* grasslands. While our results need to be framed in the context of the ecosystem and soils studied, our study provides novel insights into the effects of shrub encroachment on extracellular soil enzymes, and emphasizes the need of conducting studies in multiple geographic regions undergoing transitions to shrublands when testing the generality of soil responses to this phenomenon.

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Appendix. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.soilbio.2011.04.023.

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