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REGULAR ARTICLE

Disturbance of the biological soil crusts and performance of *Stipa tenacissima* in a semi-arid Mediterranean steppe

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Abstract Biological soil crusts (BSC) play a major role in water and nutrient fluxes in semi-arid and arid areas, affecting the establishment of vascular plants and contributing to the spatial arrangement of vegetated and open areas. However, little is known regarding their effects on the performance of extant vegetation. By using experimental manipulations (surface soil cutting and herbiciding), we evaluated the effect of the physical structure and the biotic component of smooth biological soil crusts on soil moisture dynamics, and on the nutrient and water status, growth rate, and reproductive effort of *Stipa tenacissima* tussocks in a semi-arid steppe. Soil moisture content was weakly reduced after cutting the soil surface and was not affected by herbicide application. Cutting and herbiciding the biological soil crust had no effect on most morpho-functional and reproductive traits measured in *S. tenacissima* tussocks. The integrated water use efficiency of this species, as measured by ^{13}C natural enrichment, decreased when the biotic community of biological soil crusts was killed with herbicide. In the *S. tenacissima* steppe studied, killing the BSC and breaking the continuity of the structural crust had a weak effect on the short-term performance of this species, but our results suggest that BSC exert a control on slope hydrology beyond that provided by physical soil crusts.

Keywords Cyanobacteria · Disturbance · Runoff · Soil moisture · Source-sink dynamics · Spatial heterogeneity

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Introduction

Arid and semi-arid ecosystems are characterized by a sparse vegetation cover, which is often arranged into distinctive spatial patterns (see Tongway et al. 2001 and references therein). In the absence of major disturbances, this spatial patterning may be reinforced by interactions between vegetated and open patches (Lefever and Lejeune 1997; von Hardenberg et al. 2001; Rietkerk et al. 2002). Open and vegetated patches interact in many ways, including the transport

of propagules and resources such as water, sediments and nutrients, from the former to the latter (Shachak et al. 1998; Tongway and Ludwig 2001). The limited ability of open areas to retain resources as compared to vegetated patches is a direct consequence of the lower roughness and macroporosity, and higher bulk density of the surface soil (Ahuja and Schwartzendruber 1992; Belnap 2006). These differences arise from various causes, including the capacity of vascular plants to modify environmental conditions by means of root activity, litterfall accumulation, dust interception, changes in hydrological fluxes, and the interaction with other components of the community (Whitford 2002; Cortina and Maestre 2005), and the presence of embedded rock fragments, physical crusts and biological crusts in the open areas (Poesen and Lavee 1994; West et al. 1992; Belnap 2006).

Biological soil crusts (hereafter BSC) are the community of organisms living on the soil surface in dry areas with sparse cover of vascular plants. They can be smooth, rugose, rolling and pinnacled and this structure is a key determinant of hydrological function (Belnap 2001, 2006). Smooth BSC dominated by cyanobacteria, mosses and lichens are common in arid and semi-arid environments worldwide (Belnap and Lange 2001), and may reduce water infiltration rate and increase runoff production (Kidron et al. 1999; Maestre et al. 2002; Belnap 2006). In these areas, it is difficult to identify to what extent the effect of BSC on resource flow and availability is a consequence of BSC forming organisms, or is rather caused by the structural crust underlying the BSC community (Belnap 2001, 2006). Structural soil crusts are characterized by in situ rearrangement of particles with no lateral movement, and may be reinforced by algae and fungi (Valentin and Bresson 1992). The distinction between the effect of BSC organisms, on the one hand, and the effect of a strengthened structural soil crusts, on the other, is relevant to understand the role of BSC in ecosystem functioning and determine the importance of the BSC in community assemblage.

Despite the important role that BSC play in the fluxes of water, carbon and nutrients in drylands (Johansen 1993; Belnap and Lange 2001), information on the effect of BSC on the performance of vascular plants and community dynamics is relatively scarce. Most studies on this topic have focused on early stages of their life cycle, particularly germination and early survival (e.g. Escudero et al. 2007 and references therein). But, to our knowledge, very few studies are available on the effects of BSC on established vegetation. In addition, most of these are based on comparisons between vascular plants established on areas with contrasting BSC density (Harper and Belnap 2001; DeFalco et al. 2001), but manipulative experiments are lacking.

In a recent modeling exercise, Gilad et al. (2004, 2007) suggested that a strong contrast in infiltration rates between open areas and vegetated patches together with a restricted rooting area allowed a significant increase in soil moisture availability under vegetated patches. This increase may ultimately lead to the establishment of other plants and to a general increase in plant cover and biodiversity (Aguiar and Sala 1999). In Gilad et al. (2004, 2007) study, plant patches were very sensitive to changes in infiltration rates, as their cover was substantially reduced by disturbances favoring water infiltration in open areas. Low infiltration rates have been reported for soils colonized by smooth BSC compared to soils underneath vascular plants, thus BSC may facilitate plant growth by increasing the runoff reaching vegetated patches (Maestre et al. 2002; Belnap 2006). Therefore, BSC could play a major role in sustaining the functionality of patches covered by vascular plants (Eldridge et al. 2002).

Steppes of the tussock grass *Stipa tenacissima* L. are one of the most representative vegetation types of the semi-arid areas of the Mediterranean basin, where they cover more than 32 000 km^2 (Le Houérou 1995). On gentle undisturbed slopes, *S. tenacissima* tussocks collect water and sediments from upslope open areas (Cerdà 1997; Puigdefábregas et al. 1999), and modify the microclimate of their surroundings (Maestre et al. 2001). In addition, soils underneath *S. tenacissima* tussocks show higher infiltration rates, soil organic matter and nitrogen content, lower bulk density, and are commonly deeper than soils in adjacent areas devoid of vascular plants (Cerdà 1997; Puigdefábregas et al. 1999; Maestre et al. 2001). Because of the improvement in microhabitat conditions, tussocks assemble a higher diversity of annual plants than open areas (Sánchez 1995), facilitate the establishment of perennial plants (Maestre et al. 2001; García-Fayos and Gasque 2002; Maestre et al. 2003), and sustain a BSC community dominated by mosses (Maestre and Cortina 2002).

Conversely, intertussock areas are covered by cyanobacterially-dominated BSC, which commonly

overlays structural crusts formed by microaggregates derived from slaking of large aggregates after raindrop impact (Boix-Fayos et al. 1998). *Stipa tenacissima* dependence on intertussock BSC is, however, unknown. In order to fill this gap, we evaluated the effect of (1) suppression of the BSC community and (2) alterations of the physical structure associated with smooth BSC on the performance of *S. tenacissima* tussocks in a semi-arid steppe in SE Spain. To our knowledge this is the first study aiming to differentiate the direct effects of BSC organisms from those of the structural soil crust (sensu Valentin and Bresson 1992) underlying them. We tested two hypotheses: (i) BSC control the flux of resources from open to vegetated patches and thus BSC disturbance will have a strong effect on the performance of *S. tenacissima*, and (ii) structural crusts control water fluxes independently of the presence of the BSC organisms. Therefore, *S. tenacissima* response to BSC disturbance will be stronger when both, the physical structure of the BSC and the BSC community itself, are altered.

Materials and methods

Study area

The study was carried out in a *S. tenacissima* steppe located in Relleu (SE Spain; UTM coordinates: 735591 E, 4269506 N), at an altitude of 395 m a.s.l., on a 11° slope facing SE. Climate is Mediterranean semi-arid, with mean annual precipitation and temperature of 388 mm and 16°C, respectively (1960–1990 normal period). The soil is Lithic Calciorthid (Soil Survey Staff 1994) loamy-silty loam, derived from marls and limestone (Ruiz 1993). They are shallow and highly carbonated. The steppe is dominated by *S. tenacissima* and the shrub *Rosmarinus officinalis* L., with isolated patches of the perennial grass *Brachypodium retusum* Pers. (Beauv.), and the shrubs *Rhamnus lycioides* L. subsp. *lycioides*, *Globularia alypum* L., *Anthyllis cytisoides* L. and *Osyris lanceolata* Hochst. and Steud. Average vascular plant cover is 47%. Areas devoid of vascular plants are covered by a BSC with several species of cyanobacteria (including *Microcoleus steentrupii*, *Leptolyngbya boryanum*, *L. foveolarum*, *Oscillatoria* sp., *Phormidium* sp., and *Chroococcidiopsis* sp.; Maestre et al. 2006), and lichens such as *Psora decipiens*, *Psora crenata*, *Collema* sp. and

Placidium sp. (Vicent Calatayud, Fundación CEAM, pers. comm.).

Experimental design

In January 2003 we established twenty 1.25×0.8 m^2 plots in open areas (i.e., areas covered by BSC, where vascular plants were absent), with the largest axis oriented towards the prevailing slope (hereafter, "Open" plots), and 20 plots of the same size and arrangement, but with a *S. tenacissima* tussock located at the downslope edge of the plot (hereafter, "Tussock" plots). Tussocks covered approximately one fourth of the plot surface area, whereas the rest of the plot was dominated by BSC with almost no vascular plants present. Plot perimeter was closed by inserting a 10 cm transparent plastic sheet 5 cm into the soil, with an open end located downslope of the plot and connected to a runoff storage container (Appendix I). Then we applied two treatments to randomly selected plots of each type, in a full factorial design with 2 fixed factors (BSC fragmentation and herbicide application), and 2 levels each (presence/absence). BSC was fragmented by cutting the surface crust with a sharp blade to a depth of 2 cm, creating a grid pattern with a 5×5 cm cell size oriented parallel and perpendicular to the main slope. BSC were killed by hand spraying a 0.075 g m^{-2} dose of simazine (2-chloro-4,6-bis [ethylamino]-s-triazine) dissolved in water (Zaady et al. 2004). The same treatments were repeated in November 2003. Tussocks were protected from the herbicide by covering them with a polyethylene bag during application although direct assimilation by leaves is low. A systemic effect of the herbicide on *S. tenacissima* was unlikely, as the dose used was relatively low and no rain fell for several days after herbicide application. In addition, the herbicide had no significant effect on seed germination in spring 2003 (N. Martín, unpubl. data) despite the fact that seedlings are more sensitive to this herbicide than adults (Knuteson et al. 2002). Immediately after the treatments were applied, all plots were covered with a 1×0.5 cm nylon mesh located 50 cm above the surface soil (PAR reduction 10%), to reduce raindrop kinetic energy, and thus avoid an early formation of a physical crust (in plots where it was fragmented), and provide homogeneous experimental conditions for all plots.

The efficiency of herbicide application was evaluated in January, June, August and November 2004 by

measuring Acetylene Reduction Activity (ARA) on 32 neighboring 0.5×0.5 m microplots covered by BSC and receiving the same treatments as the experimental plots (8 microplots per treatment). ARA, a surrogate of nitrogen fixing activity, has been directly related to BSC abundance and activity in several studies, including *S. tenacissima* steppes, where cyanobacteria frequently dominate (Maestre et al. 2006). On each sampling date, a 9-cm diameter Petri dish was inserted into the soil, and carefully removed to extract a 2-cm depth unaltered soil surface sample. ARA analyses were then conducted as described in Maestre et al. (2006). Data are presented as the rates of C_2H_4 accumulation over time, accounting for BSC area.

Microclimate monitoring

We measured soil temperature and moisture content by means of water-proof diodes and using the Time Domain Reflectometry method (TDR, Topp and Davis 1985), respectively. Diodes were placed at 5 cm depth in the same plots where ARA was measured (i.e, only open areas), and temperature was measured by using a modified multimeter on 7 sampling dates between December 2003 and July 2004. TDR probes, 8 cm long, were inserted vertically on the surface soil 20 cm, 50 cm and 75 cm downslope from the upper edge of each Open and Tussock plot (hereafter "Location"). Volumetric soil moisture content was measured on 8 sampling dates between May 2003 and July 2004. We used a Tektronix 1502C metallic TDR cable tester (Tektronix, Beaverton, Oregon, USA), and a site-specific calibration factor for the soils of the study area.

Stipa tenacissima response

We used predawn water potential, predawn and midday chlorophyll *a* fluorescence of PS(II), photosynthetic rate, the concentrations of foliar N, ^{13}C, and ^{15}N, growth rate and reproductive effort to evaluate the response of *S. tenacissima* tussocks to the experimental treatments. Predawn water potential was determined on one excised leaf per tussock on five sampling dates between June 2003 and May 2004 by using Scholander's pressure bomb (Soil Moisture Equipment Corp., Santa Barbara, CA, U.S.A.). We measured maximal PS(II) photochemical efficiency in dark-adapted intact leaves on 6 sampling dates

between June 2003 and August 2004 by using a portable, pulse-modulated fluorometer (PAM-2000, Walz, Effeltrich, Germany), equipped with a leaf clip holder (2030-B, Walz). Predawn and midday measurements of maximal (F_m) and minimal (F_0) fluorescence were used to calculate the maximum efficiency of the photosynthetic energy conversion of PSII ($F_v/F_m = [F_m - F_0]/F_m$), where F_v is the variable fluorescence (Genty et al. 1989). Net CO_2 assimilation rates were measured on 3 leaves per tussock and sampling date with a portable infrared gas analyzer (LI-6200; Li-COR Inc., Lincoln, NE) as described in Long et al. (1996). Measurements were taken in early morning (09:00–10:00 GMT) and midday (12:00–13:00 GMT), on January 20th 2004 and May 19th 2004. Incident PAR was adjusted and maintained at average values for each sampling period (from 850 to 1 100 μmol quanta m^{-2} s^{-2} in winter; from 1 300 to 1 950 μmol quanta m^{-2} s^{-2} in spring). On June 10th 2004 we sampled 2 healthy unshaded leaves from five *S. tenacissima* tussocks per treatment. Plant material was air-dried at 65°C until constant weight, and ground to fine powder in a ringmill. Milled samples were transferred into tin capsules containing 2 mg of sample and injected into an elemental analyzer coupled to an isotope ratio mass spectrometer (Europa Hydra 20/20, PDZ Europa, Rudheat, UK). The $\delta^{13}C$ and $\delta^{15}N$ values of the samples were calculated by using the equation $\delta X = [(R_{sample}/R_{standard})-1] \times 1,000$ (‰), where δX represents $\delta^{13}C$ or $\delta^{15}N$, and R is the mass ratio of heavy to light isotopes ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). All isotopic analyses were conducted at the Stable Isotope Facility of the University of California at Davis.

We measured the length of green leaves in 15 tillers per tussock on May 12th 2003, June 11th 2003, May 4th 2004 and May 25th 2004, and used the average difference in length between consecutive sampling dates for a given tussock and year as estimates of aboveground growth response to the experimental treatments. No significant leaf losses were detected between the sampling dates. Spikes of *S. tenacissima* grow in early spring (Haase et al. 1995), and we estimated reproductive effort as the number of spikes produced per tussock during spring of 2003 and 2004.

Statistical analyses

The effects of BSC fragmentation and herbicide on ARA activity and soil temperature in open areas were

analyzed with a three-way repeated measures analysis of variance (ANOVA), with time as the within-subject factor, and BSC fragmentation and herbicide application as fixed between-subject factors. A repeated measures ANOVA with the same structure was used to analyze the effect of BSC fragmentation and herbicide application on *S. tenacissima* chlorophyll fluorescence and predawn water potential in plots where this species was present. A five-way repeated measures ANOVA was used to evaluate the effect of time (within-subjects factor), and the presence of *S. tenacissima* tussocks, location within the plot, BSC fragmentation and herbicide application (between-subject factors) on soil moisture content. Photosynthetic rate, foliar N, ^{13}C, and ^{15}N concentrations, foliar elongation and spike production data were analyzed by using two-way ANOVA, with BSC fragmentation and herbicide application as fixed factors. Prior to analyses, we transformed foliar elongation into its decimal logarithm and foliar N and C concentrations into the function $arcsin(X)^{0.5}$ to fulfill assumptions of normality and homoscedasticity. We used Greenhouse-Geisser's epsilon to adjust the degrees of freedom in repeated measures ANOVA because the sphericity of the variance-covariance matrix could not be assumed (Mauchly's test, $p < 0.05$). The number of replicates for each analysis and combination of treatments was 5, unless noted. The degree of covariation between predawn water potential and maximal photochemical efficiency was determined by using Pearson correlation analysis. All statistical analyses were performed with the use of the SPSS 9.0 package (SPSS, Chicago, Illinois, USA).

Results

Treatment effects on BSC

Acetylene reduction rates greatly varied between sampling periods (Fig. 1). Nitrogen fixing cyanobacteria were affected by herbicide application, but not by fragmentation. Two months after treatment application, nitrogenase activity was reduced by 70% in BSC treated with herbicide, as compared to Control BSC. Differences decreased with time but they were still significant in November 2004, 1 year after the second herbicide application. The interaction between fragmen-

tation and herbicide was not significant, albeit BSC receiving both treatments consistently showed lower ARA than BSC treated with herbicide alone.

Treatment effects on soil microclimate

Soil moisture content was highly variable; the lowest values (ca. 5%) were reached during summer 2003 and summer 2004 (Fig. 2; Table 1). The absence of *S. tenacissima* tussocks and BSC fragmentation decreased soil moisture in a similar manner (average reductions of 1% in both cases; Fig. 2a and b). Herbicide application and location had a marginally significant effect on soil moisture content (Fig. 2c and d). Treatment effects were not uniform across the study period, as shown by the significant 2-way interactions involving time. Average soil surface temperature ranged from 10 to 32°C over the study period, but we found no significant effect of BSC fragmentation and herbicide application on this variable (Appendix II).

Treatment effects on *Stipa tenacissima* performance

Predawn water potential of *S. tenacissima* tussocks ranged between −0.8 and −1.7 MPa during spring, winter and autumn, but decreased to minimum values of −3.4 MPa and −6.7 MPa during summer 2003 and 2004, respectively (Fig. 3). We found no significant effect of BSC fragmentation or herbicide application on predawn water potential. Temporal variation in maximal photochemical efficiency was similar to that of water potential (Fig. 3), as both variables were highly correlated ($r = 0.830$, $P < 0.001$, $n = 104$). Under high water availability (from November 2003 to May 2004), photochemical efficiency of *S. tenacissima* showed an average value of 0.68. This value decreased in summer 2003 and, especially, in summer 2004 when it reached an average value of 0.28. No significant effect of BSC manipulation on this variable was observed.

Photosynthetic rate of *S. tenacissima* ranged between 6.3 and 9.7 μmol CO_2 m^{-2} s^{-1} in January 2004, when water availability was high, and between 1.0 and 4.0 μmol CO_2 m^{-2} s^{-1} during the May 2004 drought (Table 2). The treatments evaluated did not affect this variable. Integrated water-use-efficiency, as estimated from ^{13}C natural enrichment, ranged between −24.5 and −25.5‰, and significantly decreased in

Fig. 1 Nitrogen fixing activity (estimated as Acetylene Reduction Activity) in the surface soil of a *Stipa tenacissima* steppe as affected by the fragmentation (*F*) and herbicide application (*H*) of biological soil crusts. Bars correspond to averages and standard errors of *n*=8 plots per treatment. Results of Repeated-measures ANOVA are shown
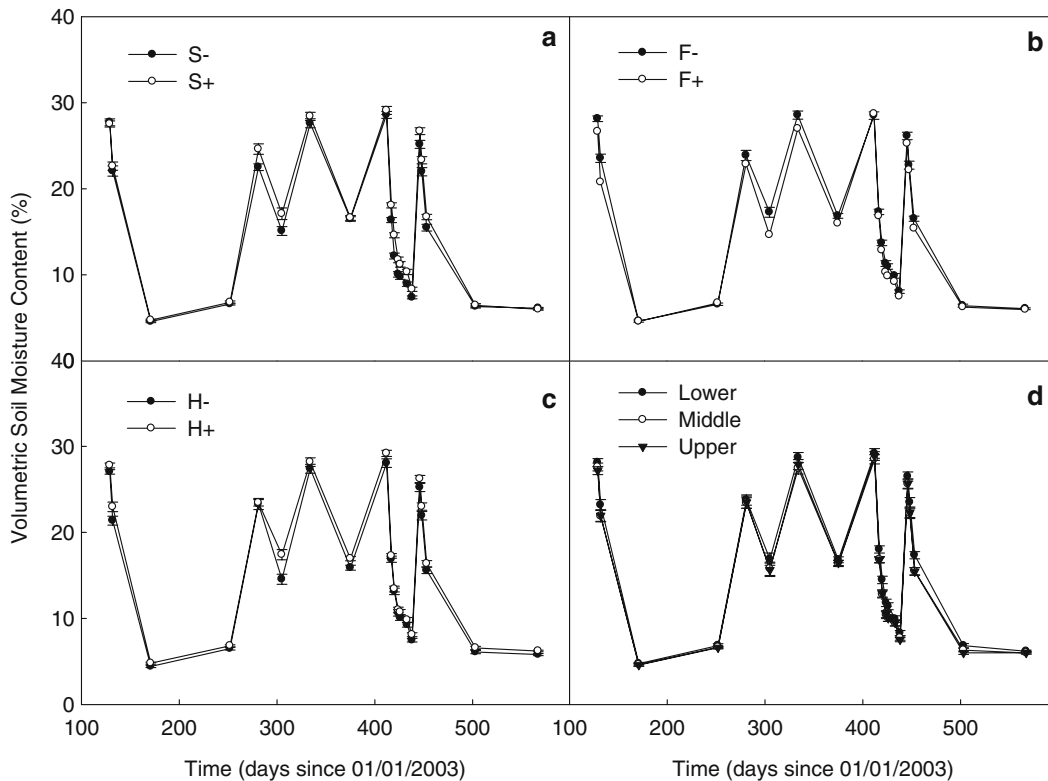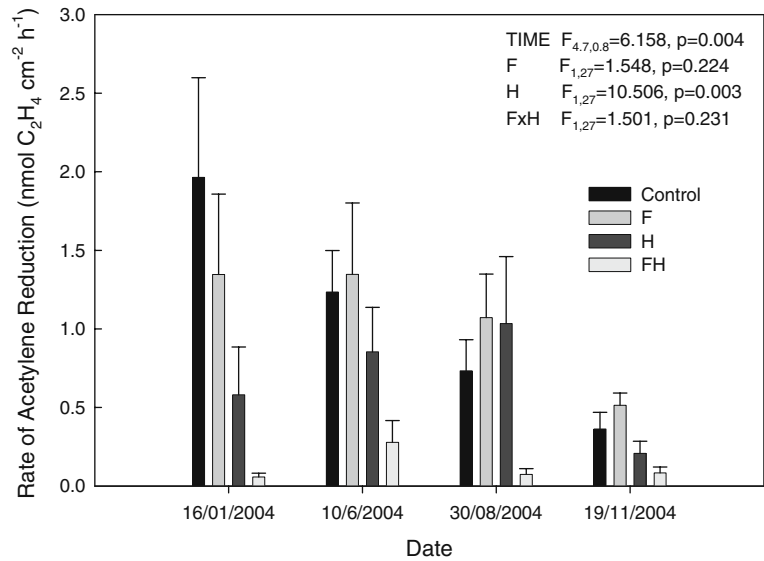

Fig. 2 Volumetric soil moisture content in the 0–8 cm depth surface soil as affected by the presence of a *Stipa tenacissima* tussock (S−/S+; **a**), biological soil crust fragmentation (F−/F+; **b**), herbicide application (H−/H+; **c**), and location within the plot (Upper, Middle and Lower sections of the plot; **d**). Bars correspond to averages and standard errors of *n*=60 (**a**, **b**, **c**) and 40 (**d**) sampling points per treatment. The complete factorial design has been split for clarity. See Table 1 for statistical analyses of these data

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Table 1 Results of a repeated-measures ANOVA to evaluate changes in soil moisture content as affected by the presence of a *Stipa tenacissima* tussock, BSC fragmentation, herbicide application and location within the microplots. Results of >2-ways interactions involving Time were not statistically significant and are not shown

Factor	F	d.f.	P
Time (T)	2 089.168	5.5, 525	<0.001
Stipa (S)	8.573	1,96	0.004
BSC fragmentation (F)	10.959	1,96	0.001
Herbicide application (H)	2.798	1,96	0.098
Location (L)	2.916	1,96	0.059
S × F	0.962	1,96	0.329
S × H	1.490	1,96	0.225
F × H	0.283	1,96	0.596
S × F × H	1.906	1,96	0.171
S × L	0.113	1,96	0.893
F × L	0.412	1,96	0.663
S × F × L	0.084	1,96	0.919
H × L	0.975	1,96	0.381
S × H × L	0.411	1,96	0.664
F × H × L	0.182	1,96	0.834
S × F × H × L	0.319	1,96	0.727
T × S	5.084	5.5,525	<0.001
T × F	4.564	5.5,525	<0.001
T × H	2.418	5.5,525	0.030
T × L	0.988	10.9,525	0.456

tussocks where the BSC had been killed with herbicide. We found no significant effect of BSC fragmentation and herbicide application on either foliar N concentration or ^{15}N enrichment.

Average leaf elongation in spring 2003 ranged from 12 to 20 cm, and showed no significant response to BSC fragmentation or herbicide application (Fig. 4). Similar results were obtained in spring 2004. The average number of spikes per tussock was substantially lower in *S. tenacissima* tussocks where the BSC had been fragmented, but differences were not statistically significant (Fig. 4). This may be a consequence of the variability in spike production found within each treatment (average coefficient of variation ca. 80%). Spike production in 2004 was much lower (average values 0.3–5 spikes per tussock), with 40% of the tussocks producing no spikes. Treatments had no significant effects on the average number of spikes produced in 2004.
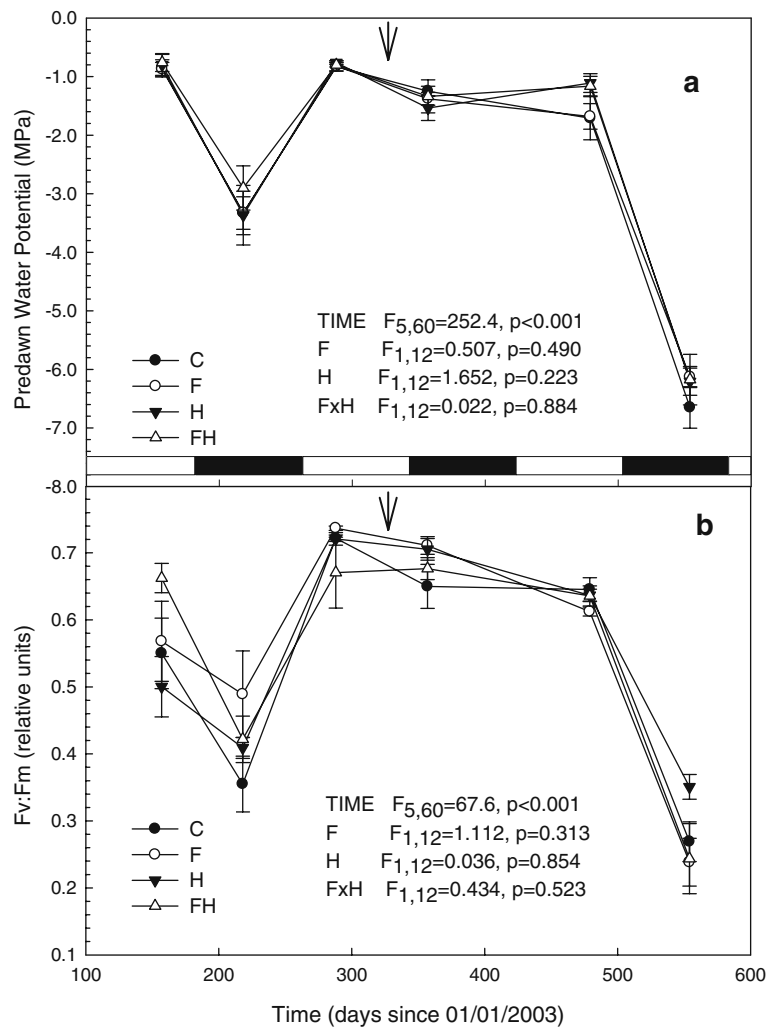
Discussion

Biological soil crusts have a strong capacity to modify soil properties and the fluxes of water, carbon, nutrients and sediments (Belnap and Lange 2001; Maestre et al. 2005). Thus, BSC-mediated effects are likely to affect the performance of established vegetation, and propagate to larger scales (Evans and Ehleringer 1993), particularly when the plant species affected play a major role in ecosystem dynamics. Following current knowledge on *S. tenacissima* steppes composition and dynamics, and the results of Gilad et al. (2004, 2007) and Eldridge et al. (2002), we advanced the hypothesis that any alteration of the BSC which increased infiltration rate and reduced runoff would negatively affect the performance of *S. tenacissima*. This hypothesis was not supported by our results, as *S. tenacissima* showed a weak response to alterations of the physical and biotic structure of BSC. Our second hypothesis, which predicted that the response of *S. tenacissima* to BSC disturbance should be stronger when the physical structure of the BSC is altered, was not supported either, as the effect of BSC fragmentation on *S. tenacissima* performance was not substantially different from the one elicited by herbicide application.

Treatment effects on BSC

The treatments applied affected BSC integrity and surface soil properties, as denoted by changes in N fixation activity, soil moisture content and water infiltration rate (data not shown). Herbicide application had a strong effect on N fixation activity, suggesting that the populations of N-fixing cyanobacteria and cyano-lichens may have been reduced for an extended period of time. Although not all BSC cyanobacteria fix nitrogen, N fixation can be used as a surrogate of BSC biomass in these soils (Maestre et al. 2006). Simazine remains active in the soil for months (García-Valcárcel and Tadeo 1999; Barra et al. 2005), and it may have precluded BSC colonization during the humid season. This is in agreement with studies showing a prolonged reduction of BSC activity in response to herbicide application (Zaady et al. 2004). In addition, we may note that early colonizers of the BSC community may take several years to establish on barren soils when water is limiting (Belnap and Eldridge 2001; Maestre et al. 2006). In the present study, herbicide effect on moss cover was visible by direct observation (ca. 50%

Fig. 3 Water status (measured as Predawn Water Potential; **a**) and photosystem II efficiency (measured as maximal photochemical efficiency, Fv:Fm; **b**) of *Stipa tenacissima* leaves as affected by the fragmentation (*F*) and herbicide application (*H*) of the biological soil crusts covering the upslope section of experimental plots. Bars correspond to standard errors of *n*=5 individuals per treatment. *Arrows* show the date of the second treatment application (November 2003). *Horizontal black* and *white* bars correspond to seasons from spring 2003. Results of repeated-measures ANOVA are shown

(a) Predawn Water Potential (MPa)

TIME $F_{5,60}=252.4$, $p<0.001$
F $F_{1,12}=0.507$, $p=0.490$
H $F_{1,12}=1.652$, $p=0.223$
FxH $F_{1,12}=0.022$, $p=0.884$

(b) Fv:Fm (relative units)

TIME $F_{5,60}=67.6$, $p<0.001$
F $F_{1,12}=1.112$, $p=0.313$
H $F_{1,12}=0.036$, $p=0.854$
FxH $F_{1,12}=0.434$, $p=0.523$

Time (days since 01/01/2003)

reduction in May 2003; N. Martín, unpubl. data). Confocal microscopy observations provided further evidence of a decrease in cyanobacterial activity after herbicide application (B. Díez, University of Alicante, pers. comm.). The trend towards a higher decrease in N fixation activity in plots where the BSC was killed and fragmented could be the result of further herbicide penetration. In contrast to herbicide application, physical alteration of the surface soil did not affect BSC ability to fix atmospheric nitrogen, which may reflect the relative small surface area affected by this treatment.

Treatment effects on soil microclimate

Soil moisture content was higher where *S. tenacissima* tussocks were present. Interestingly, we found a trend towards lower differences in soil moisture content between Open and Stipa sites as we moved downslope. Indeed, the average difference between both sites was reversed at this location (i.e., average moisture content close to the tussocks was lower than in the corresponding location in open plots; Appendix III). Our results suggest that water uptake may have balanced the positive effect of shade on moisture content near *S. tenacissima* tussocks, and thus may have been responsible for the lack of differences found in the lower section of the experimental plots. Shade and runoff concentration may compensate for higher water uptake by *S. tenacissima* when water is available, but this may not be the case when it is scarce. Under severe drought, transpiration losses may deplete water in the vicinity of the tussocks, and thus soil moisture content may be lower in those microsites

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Table 2 Photosynthetic rate, N concentration and ^{15}N and ^{13}C enrichment in *Stipa tenacissima* leaves, as affected by BSC fragmentation (F) and herbicide application (H). Data correspond to means and standard errors of $n=5$ tussocks. Values of the F statistic for the 2 factors evaluated and their interaction is shown. A significant value ($p<0.05$) is shown in bold

	Treatments				ANOVA results					
	Control	F	H	F × H	F		H		F × H	
					$F_{1,15}$	P	$F_{1,15}$	P	$F_{1,15}$	P
Photosynthetic rate (μmol CO$_2$ m^{-2} s^{-1})										
20/01/04 morning	6.3± 0.7	7.6± 0.8	9.7± 1.9	8.3± 1.1	0.045	0.835	1.849	0.194	1.485	0.242
20/01/04 midday	6.7± 1.3	6.6± 0.8	6.7± 1.1	6.9± 1.2	0.705	0.414	0.133	0.721	1.195	0.292
19/05/04 morning	1.2± 0.2	1.3± 0.3	1.7± 0.5	1.0± 0.2	0.004	0.953	0.013	0.912	0.017	0.898
19/05/04 midday	2.7± 0.3	3.2± 0.5	4.0± 0.5	3.2± 0.6	0.001	0.974	2.809	0.114	1.249	0.281
δ^{13}C (‰)	−24.8± 0.3	−24.5±0.3	−25.5± 0.3	−25.4±0.3	0.405	0.534	**6.938**	**0.019**	0.144	0.710
N (mg g^{-1})	8.8± 1.2	6.2±0.9	9.6±1.4	7.6±0.1	3.813	0.070	1.210	0.289	0.142	0.712
δ^{15}N (‰)	−1.86± 0.44	−2.68±0.56	−2.78±0.41	−2.10±0.32	0.022	0.884	0.137	0.721	2.527	0.133

than in open areas. The study area experienced an intense, although not unusual, drought during the study period. Accumulated rainfall between January 2003 and January 2004 was 199 mm, 51% of the local average, and only 14 mm fell between May 7th 2003 and October 18th 2004 (Appendix IV).

BSC fragmentation significantly decreased soil moisture content, especially in the sector of the
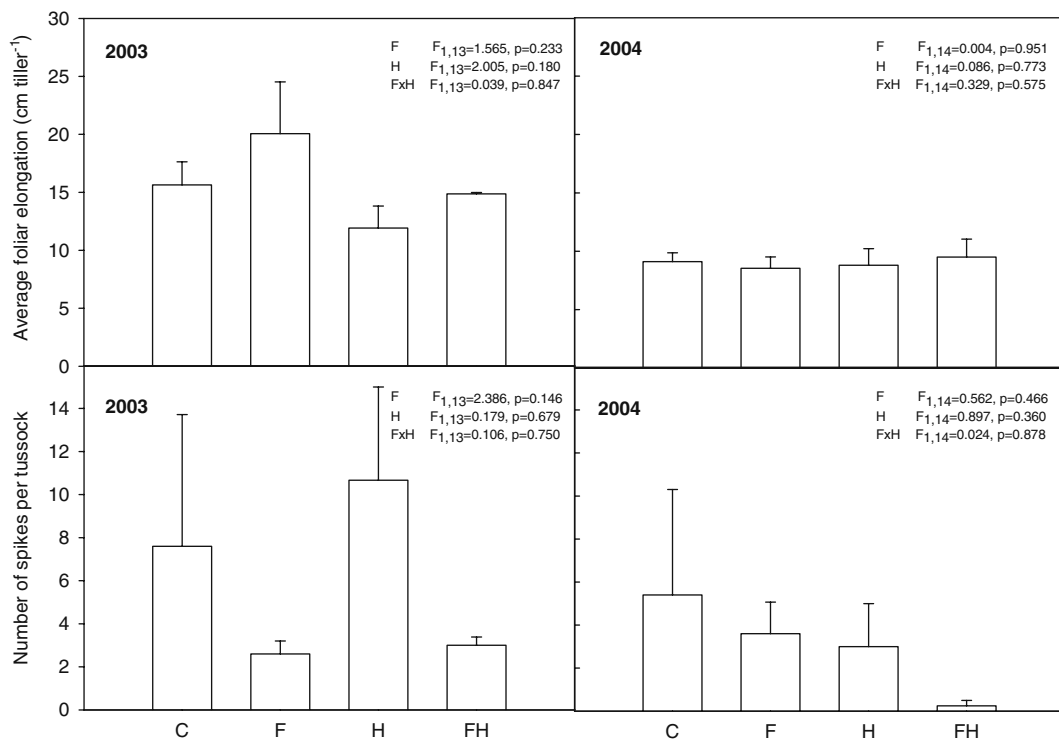
Fig. 4 Average increase in leaf length per tiller (*top*) and spike production (*bottom*) in *Stipa tenacissima* tussocks in spring 2003 and spring 2004 as affected by the fragmentation (*F*) and herbicide application (*H*) of the biological soil crust. Bars correspond to standard errors $n=4$–5 individuals per treatment. Results of the two-way ANOVA are shown

experimental plots that was farther from *S. tenacissima* tussocks. These results were unexpected, as runoff was substantially reduced in plots where the BSC had been fragmented as compared to untreated plots (e.g., an average decrease of 46% and 34% in surface runoff for open plots where the BSC had been fragmented, and fragmented plus herbicided, respectively; Martín et al. 2003). A resulting increase in evaporation rate could explain the decrease in soil moisture content after BSC fragmentation. This process is supported by studies showing a decrease in soil moisture content in tilled soils (Blevins et al. 1983; Mygdakos et al. 2005). Also, increased infiltration may have been restricted to the top soil layers which dry out very quickly. These short-term small changes in moisture availability may not be detected by periodic TDR measurements.

Treatment effects on *Stipa tenacissima* performance

The decrease in water use efficiency in *S. tenacissima* tussocks suggests that their water status improved after herbicide application, and provides support to the marginally significant effect of this treatment on soil moisture observed. This decrease was not the result of a reduction in vascular plant cover upslope of the tussocks in plots treated with herbicide, as vascular plants were absent at the beginning of the experiment, and recruitment in spring 2003 was transient and showed no effect of herbicide (N. Martín, unpubl. data). Direct effects of herbicide on *S. tenacissima* cannot be excluded, but they are unlikely, as the dose applied was relatively low. No major rainfall occurred soon after herbicide application and young plants, which are more sensitive to this herbicide, showed no effect of this treatment. Simazine applied at similar doses as the ones used in the present study had deleterious effects on smooth BSC structure, resulting in increased losses of mineral soil, organic matter and nitrogen in soils with sandy loam texture (Zaady et al. 2004). Assuming that simazine had similar effects on BSC in our study site, our results suggest that temporary BSC suppression resulted in a small increase in water availability that benefited *S. tenacissima* tussocks. The effect of BSC on water availability depends on BSC type, soil properties and rainfall regime. Thus, both hydrophobic and hydrophilic responses have been reported for BSC components (Kidron et al. 1999; Belnap 2006).

The reduction in N fixation rate in BSC treated with herbicide had no effect on either foliar N concentration or

^{15}N enrichment of *S. tenacissima*. Several studies have shown a consistent positive effect of the presence of N-fixing BSC on the N status of vascular plants (Belnap and Harper 1995; Pendleton et al. 2003). This disagreement may partly reflect the low nitrogenase activity in the study area (below 2 nmol C_2H_4 cm^{-2} h^{-1} during most of the year), and the relatively high fertility of the surface soil as compared to other *S. tenacissima* steppes (i.e., average 6.9% organic matter in the surface soil; Maestre and Cortina 2004). Zaady et al. (2004) have shown that shrubs from the northern Negev desert retain more than 50% of the NO_3-N dissolved in runoff water coming from upslope areas covered by BSC. They also found that suppression of BSC with herbicide resulted in a strong increase in nitrate export. The magnitude of the fluxes, however, was very low and unlikely to generate major changes in the nutritional status of vascular plants. BSC fragmentation promoted a marginally significant reduction in foliar N concentration. This reduction may not be related to BSC activity as it was not accompanied by changes in N fixation rate or ^{15}N enrichment. Overall, our results suggest that the role of BSC in the nutritional status of vascular plants in *S. tenacissima* steppes is less significant than in other dryland areas (Evans and Ehleringer 1993).

The direct effects of the BSC on *S. tenacissima* water and nutrient status were weak, suggesting that the functional role of BSC is less significant than the role played by structural soil crusts and resource-modulating organisms such as vascular plants and soil fauna. Nevertheless, we must bear in mind that only the short-term effects of BSC alteration were assessed in this study. We cannot exclude the possibility that a cumulative effect of BSC suppression on water and nutrient fluxes could have a stronger impact on *S. tenacissima* performance over a longer term. In addition, BSC impact on soil surface properties may persist for more than 2 years after their death, as observed under vascular plants (Kelly and Burke 1997, Martinez-Mena et al. 2002, Armas et al. 2008). Finally, the magnitude of the water pulses during the period of study may have been too small to trigger *S. tenacissima* response (Schwinning and Sala 2004).

Conclusion

Alterations of the physical structure and the BSC community, such as the ones applied in our study, had

a limited effect on short-term *S. tenacissima* performance. The fragmentation of the BSC had a weak negative effect on soil moisture availability, probably as a result of increased evaporation. But this change was not strong enough to affect *S. tenacissima* water and nutrient status, growth rate or reproductive output. On the other hand, killing the BSC with herbicide significantly reduced water use efficiency of this species but did not affect other response variables. Our results suggest that BSC exert a direct control of slope hydrology beyond that provided by physical soil crusts, but severe alterations of the structural crust or the BSC may be needed to elicit a strong response of *S. tenacissima* tussocks.

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