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Ecosystem structure and soil-surface conditions drive the variability in the foliar δ^{13}C and δ^{15}N of *Stipa tenacissima* in semiarid Mediterranean steppes

Abstract We evaluated the effects of ecosystem composition and structure (species richness and diversity, cover and spatial attributes of vegetation), abiotic factors (climate and topographical features) and the condition of the bare-ground areas (evaluated using soil-surface indicators) on the performance of *Stipa tenacissima* [evaluated using foliar δ^{13}C, δ^{15}N, nitrogen concentration and the carbon-to-nitrogen (C:N) ratio] in 15 steppes of SE Spain. Foliar δ^{13}C values of *S. tenacissima* showed a low degree of variation in the studied steppes, with average values ranging from −24.1 to −22.9‰. Higher variation was found in the δ^{15}N values, which ranged from −5.5 to −2.4‰. The nitrogen concentration and the C:N ratio varied between 5.0 and 8.0 mg g^{-1}, and between 55.4 and 85.3, respectively. The δ^{13}C values became less negative with increasing spatial aggregation of perennial vegetation, while the C:N values increased with increasing perennial vegetation cover. The δ^{15}N values became more negative with increasing infiltration in the bare-ground areas, but the nitrogen concentration was not related to any of the environmental variables measured. Our results suggest that the relative importance of ecosystem structure and soil-surface conditions in the bare ground areas was higher than that of abiotic factors as determinants of the performance of *S. tenacissima*. The results also show that even subtle changes in these ecosystem features may lead to modifications in plant performance in semiarid *S. tenacissima* steppes, and thus to modifications in the associated ecosystem functions in the mid- to long-term.

Introduction

Inspired by an increasing concern about the ecological impacts and consequences of global change, the question of how the structure and composition of communities affect ecosystem productivity, processes, and services has gained renewed interest (Loreau et al. 2002; Garnier et al. 2004). Drastic changes in ecosystem structure and composition, like those associated with land-use changes such as deforestation and cultivation, have profound consequences for ecosystem processes and services such as nutrient dynamics, carbon sequestration, soil conservation and air-quality control (Cerdà 1997; Mosier 1998; Guo and Gifford 2002). Less apparent changes in ecosystem composition and structure, like those associated with human-induced grazing and erosion (Adler et al. 2001; Jauffret and Lavorel 2003), also promote important modifications in plant performance and associated ecosystem processes (Papatheodorou et al. 1993; García-Fayos et al. 2000).

The effects of changing ecosystem structure and functioning on plant performance can be of major relevance in arid and semiarid ecosystems, which are often arranged as a two-phase mosaic of vegetated patches inserted on a bare-ground matrix (Valentin et al. 1999). In these ecosystems, the maintenance of vegetated patches, and thus the overall functioning of the ecosystem, is largely dependent on the redistribution of water, sediments and nutrients from the bare-ground areas to the discrete plant patches (Noy-Meir 1973; Aguiar and Sala 1999). These source–sink dynamics depend on complex interactions between climate, topography, vegetation and soil-surface properties. Rainfall amount and frequency is a major driver of plant performance in arid and semiarid areas (Reynolds et al. 2004), and both rainfall attributes play a

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prevalent role in the dynamics of water redistribution in these areas (Bergkamp et al. 1999). However, such redistribution is also influenced by topographical features (Puigdefábregas et al. 1999), by ecosystem structural attributes such as the number, width and spatial pattern of discrete plant patches (Ludwig and Tongway 1995), and by the soil-surface conditions in the bare-ground areas (Eldridge et al. 2000; Maestre et al. 2002). Thus, any reduction in the runoff fluxes reaching the plants promoted by changes in ecosystem structure or in soil-surface conditions in the bare-ground areas—such as those promoted by anthropogenic disturbance (Alados et al. 2004; Maestre and Cortina 2004)—may negatively affect plant performance (Seghieri and Galle 1999; Puigdefábregas et al. 1999). Such a negative effect may, in the long-term, modify ecosystem structure, impair ecosystem functionality and promote degradation and desertification processes (Aguiar and Sala 1999; von Hardenberg et al. 2001; Reynolds and Stafford Smith 2002).

In arid and semiarid environments, variables related to the use of water and nutrients are especially important to assess plant performance, especially in the case of long-lived perennial plants (Whitford 2002). To this end, the use of stable carbon and nitrogen isotope ratios (δ^{13}C and δ^{15}N, respectively) to study the acquisition and use of resources by plants has become a basic tool for terrestrial ecologists (Lajtha and Michener 1994; Högberg 1997; Dawson et al. 2002). The δ^{13}C of leaves has often been used as an index of plant water-use efficiency, defined as the ratio between photosynthesis per unit leaf area and stomatal conductance, and of integrated photosynthetic activity throughout the period the leaf tissue was synthesized (Farquhar et al. 1989; Dawson et al. 2002). Despite the fact that the interpretation of the δ^{15}N of leaves is not straightforward (Handley et al. 1999; Evans 2001), this variable can provide plant ecologists with useful information on the nitrogen sources used by plants, especially when the nitrogen supply rate is low in relation to plant demand (Högberg 1997).

In this study, using leaf δ^{13}C, δ^{15}N, nitrogen concentration and the carbon-to-nitrogen ratio (C:N), we evaluated the effects of ecosystem composition and structure, abiotic factors and the condition of the soil surface in the bare-ground areas on the performance of the tussock grass *Stipa tenacissima* L. Open steppes dominated by this species constitute one of the major vegetation types in the driest areas of the Mediterranean Basin, and result from the degradation of Mediterranean open forests and shrublands in N Africa and SW Europe, respectively (Rivas Martínez 1987; Le Houérou 2001), by human activities such as grazing, harvesting, and repeated burning, which have been occurring in the area for millennia (Barber et al. 1997). *S. tenacissima* steppes are good model systems in arid land ecology, as the vegetation patterns and the processes governing their functioning resemble those described in arid and semiarid regions throughout the world (Puigdefábregas et al. 1999; Valentin et al. 1999). Our main objectives were (1) to evaluate if there is any relationship between plant performance and ecosystem composition and structure, abiotic factors

and soil-surface conditions in the bare-ground areas and, if so, (2) to assess the relative importance of these factors as drivers of plant performance. Our hypothesis was that changes in ecosystem structure and soil-surface conditions leading to a modification in the runoff fluxes reaching *S. tenacissima*, either by reducing plant cover, by modifying the spatial pattern of vegetation or by altering the infiltration dynamics of the bare-ground areas, will promote a decrease in the plants' performance of *S. tenacissima* (Puigdefábregas and Sánchez 1996).

Materials and methods

Study area

Our study was conducted at 15 experimental sites located in the provinces of Alicante and Murcia, in SE Spain (Fig. 1). Site selection aimed to capture a significant range of the variability in average rainfall in semiarid *S. tenacissima* steppes, and to reduce between-site variability associated with vegetation type, slope aspect and soil type. The soil in all sites is a Lithic Calciorthid (Soil Survey Staff 1990). The climate is Mediterranean semiarid, with average annual precipitation ranging from 220–388 mm, and average annual temperature ranging from 16–18°C (1960–1990 period). Topographical features, such as elevation, slope and aspect, also vary between sites (ranges 63–769 m, 11–28° and 140–290° for elevation, slope and aspect, respectively). The vegetation is in all cases an open steppe dominated by *S. tenacissima*, but total plant cover, perennial plant species richness and diversity vary sub-

Fig. 1 Map of the study area showing the location of the 15 study sites (denoted as a two letter code) and of some cities in the provinces of Alicante and Murcia (SE Spain)

stantially [ranges 19–47%, 16–36 and 0.7–2 bits for cover, species richness and diversity (Shannon's H-index), respectively]. See Maestre (2004) and Maestre and Cortina (2004) for a detailed account of the study sites.

Stable isotope analyses

At each site, six individual mature *S. tenacissima* tussocks of homogeneous size were randomly selected for isotopic ($\delta^{13}C$ and $\delta^{15}N$) and elemental (total carbon and nitrogen) analyses. Healthy sunny and shaded leaves were randomly collected from the upper part of the canopy of each tussock (25–40 leaves were bulked for each tussock) in July 2002, during the peak of summer drought. There are no previous studies evaluating the seasonal variation in the isotopic composition of *S. tenacissima*. The physiological ecology of this species resembles that of pohiquilohydric plants (Balaguer et al. 2002), and it quickly responds to increases in soil water availability by increasing photosynthetic activity, leaf growth and nitrogen uptake (Pugnaire et al. 1996; Haase et al. 1999). We decided to sample during the peak of summer drought to minimize the effects of antecedent rainfall events on the isotopic and nutrient concentration of *S. tenacissima*, and thus to allow a better assessment of our objectives. Collected plant material was air-dried at 65°C until constant weight, and was later ground to a fine powder in a ring mill. 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 using $\delta X = [(R_{sample}: R_{standard})-1] \times 1{,}000$ (‰), 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 $^{15}N/^{14}N$). The precision across runs during the time period in which samples were analyzed (sensu Jardine and Cunjak 2005) was estimated using replicated measurements of standards (ammonium sulfate for $\delta^{15}N$ and sucrose for $\delta^{13}C$). It was -23.85 ± 0.032‰ and -1.33 ± 0.152‰ for $\delta^{13}C$ and $\delta^{15}N$ measurements, respectively (mean $\pm$ SD; $n = 19$). The precision within runs (sensu Jardine and Cunjak 2005) was estimated using two sets of duplicated measurements of finely ground peach leaves (National Institute of Standards and Technology SRM 1547). The $\delta^{13}C$ values obtained for each run were -26.02 ± 0.021‰ and -26.06 ± 0.000‰ (mean $\pm$ SD; $n = 2$). The $\delta^{15}N$ values obtained for each run were 1.97 ± 0.148‰ and 1.88 ± 0.049‰ (mean $\pm$ SD; $n = 2$). All the isotopic analyses were conducted at the Stable Isotope Facility of the University of California at Davis.

Assessment of ecosystem abiotic, structural and compositional attributes

For this study, we focussed on the abiotic, structural and compositional attributes that determine water fluxes and nutrient inputs in *S. tenacissima* steppes. At each site we obtained the following abiotic variables: elevation, slope, aspect, Universal Transverse Mercator (UTM) co-ordinates (north and east) and mean annual rainfall at the nearest meteorological station (period 1960–1990). To characterize the structure and composition of the ecosystem, we established 30×30 m plots at all the sites. In these plots we measured the total area covered by isolated late-successional sprouting shrubs (*Pistacia lentiscus*, *Quercus coccifera*, *Rhamnus lycioides*, *Ephedra fragilis*, *Erica multiflora* and *Juniperus oxycedrus*) and the number of perennial plant species (see Maestre and Cortina 2005, for a complete checklist), which was used as our estimate of species richness. Four parallel 30-m transects, separated by 8 m each, were established to measure the total cover (%), number (number/10 m of linear transect) and width perpendicular to the transect (m/10 m of linear transect) of discrete perennial plant patches (including sprouting shrubs), as described in Maestre and Cortina (2004). Transects were also used to evaluate perennial plant species diversity (Shannon's index, Greig-Smith 1983) and spatial pattern by using the line-point sampling method, with a sampling frequency of 50 cm along the 30-m transect. We characterized the spatial pattern of perennial vegetation by using the spatial analysis by distance index (SADIE) methodology (Perry 1995) and the data generated from the diversity survey. Details of this approach can be found elsewhere (Perry et al. 1999, 2002), and will not be given here. We used the SADIE index of aggregation (I_a) to summarize the spatial pattern of perennial vegetation at the transects; it was clumped if $I_a > 1$, random if I_a was close to 1, and regular if $I_a < 1$ (Perry et al. 1999). An increase in the I_a values suggests an increase in the spatial aggregation of vegetation. We performed SADIE analysis separately for each transect using presence/absence data and the SADIEShell for Windows software, version 1.22 (downloaded from http://www.iacr.bbsrc.ac.uk/pie/sadie). All the SADIE analyses were performed with 2,340 permutations.

We also measured the condition of the soil surface in the bare-ground areas using the "landscape function analysis" methodology (LFA; Tongway 1995; Tongway and Hindley 2004). LFA summarizes the condition of the soil surface via three quantitative indices: (1) stability or resistance to erosion, (2) infiltration/water holding capacity and (3) nutrient cycling. These indices provide information, respectively, on the ability of the soil to withstand erosive forces, infiltrate rainfall water and cycle organic matter back into the soil. The indices, which are measured as percentages, are strongly related to quantitative measures of ecosystem processes such as infiltration rate, aggregate stability and soil respiration (Tongway and Hindley 2003), key aspects used to define ecosystem functioning in arid and semiarid areas (Whitford 2002). Field data for obtaining these indices were gathered in 0.5×0.5 m squares (16 per 30×30 m plot) randomly placed in areas devoid of vascular plants. In these squares we measured 11 soil-surface indicators (soil cover, basal cover of perennial grasses and shrub canopy cover, litter cover, biological crust cover, crust brokenness, erosion features, deposited materials, soil microtopography, surface nature, slake test for soil stability and surface texture) following the guidelines of Tongway and Hindley 2004. These were further combined to obtain the three LFA indices with a Microsoft

Excel template developed by David Tongway (http://www.cse.csiro.au/research/ras/efa/index.htm). See Maestre and Cortina (2004) for further details on the methodology and calculations regarding the LFA indices.

Statistical analyses

As the $\delta^{13}C$, $\delta^{15}N$, nitrogen concentration, and C:N values of each *S. tenacissima* tussock were evaluated in the same sample, and thus these measurements cannot be considered to be independent of each other, multivariate analysis of variance (MANOVA) was used to look for overall site effects on these variables. This analysis was followed by univariate ANOVA and the Tukey's *b*-test for post hoc comparisons. Prior to these analyses, some data were transformed to achieve homogeneity of variances in their distribution. $\delta^{13}C$ and C:N data were transformed with a power ($x^{4.5}$) function and a logarithmic function, respectively. Even after these transformations, our data set did not meet the homogeneity of variance–covariance matrices assumption (Box's *M*-test, $F = 1.68$, df = 140, 4,053, $P < 0.001$). Despite this, we conducted the MANOVA using the Pillai's trace statistic, which is robust to deviations from this assumption, especially when the sample sizes are equal (Quinn and Keough 2002).

We formed three matrices with the compositional–structural, abiotic and soil-surface condition data obtained from our study sites. Seven variables were used to characterize the composition and structure of the study sites (composition–structure matrix): area covered by sprouting shrubs, perennial species richness, diversity, cover, width perpendicular to the transect, number of patches and spatial pattern. For the latter five variables, each site was characterized by the average value obtained from the four transects. The abiotic environment of the study sites was characterized with six variables (abiotic matrix): elevation, slope, aspect, UTM coordinates (north and east), and mean annual rainfall at the nearest weather station. Soil-surface conditions in the bare-ground areas were characterized in each site as the average value of each of the three LFA indices (stability, infiltration and nutrient cycling). These three variables constituted our soil-surface matrix.

Due to the low number of sites as compared to the large number of variables forming the composition–structure, abiotic and soil-surface matrices, we reduced the dimensionality of these matrices. First, we eliminated those variables that were strongly correlated (and thus were statistically redundant; García 2004). A correlation matrix of all the variables forming the composition–structure, abiotic and soil-surface matrices is shown in Appendix 1. Species richness was significantly correlated with elevation ($r = 0.866$), the UTM north coordinate ($r = 0.807$) and the area covered by sprouting shrubs ($r = 0.786$). The latter variable was significantly correlated with elevation ($r = 0.786$). The total cover of perennial plant patches was also highly correlated with their width ($r = 0.915$). Thus, we removed species richness and the width of patches from the composition–structure matrix, and elevation from the abiotic matrix. We further reduced the dimensionality of the composition–structure, abiotic and soil-surface matrices by using principal component analysis (PCA) with a correlation matrix and Varimax rotation. From each PCA, we retained all the components that had eigenvalues greater than 1 (Quinn and Keough 2002). The relationships between each extracted component and the variables forming each matrix were evaluated using correlation analyses. As some of the variables forming the composition–structure and abiotic matrices were non-normally distributed (Kolmogorov-Smirnoff test, $P < 0.05$), significance of the correlation coefficients for these matrices was determined using a permutation test with 10,000 randomizations (Legendre and Legendre 1998).

To evaluate the importance of the composition–structure, abiotic and soil-surface matrices as determinants of isotopic ($\delta^{13}C$ and $\delta^{15}N$) and nutrient (nitrogen concentration and C:N) data, we used multiple linear regressions with a stepwise procedure selection (significance level = 0.05). In these regressions, we used the plant performance data as dependent variables (average values for each site) and all the extracted components from the PCAs as independent variables. Separate regressions were conducted for each of the performance variables evaluated. Prior to multiple regression analyses, we checked for collinearity between the extracted PCA components. We performed multiple linear regressions separately for each component using it as the dependent variable, and the rest as the independent variables, and used the variance inflation factor (VIF) between the different components as an indicator for collinearity. The VIF was in all cases below six, suggesting the absence of strong collinearity problems (Chatterjee and Price 1991). All statistical analyses were performed with the software SPSS for Windows 9.0 (SPSS, Chicago, IL, USA).

Results

Foliar $\delta^{13}C$ values of *S. tenacissima* showed a low degree of variation in the studied steppes, with average values ranging from −24.1 to −22.9‰ (Fig. 2). Higher variation was found in the $\delta^{15}N$ values, which ranged from −5.5 to −2.4‰. The nitrogen concentration and the C:N ratio varied between 5.0 and 8.0 mg g^{-1}, and between 55.4 and 85.3, respectively. We found an overall significant site effect on all the plant performance variables measured (MANOVA: Pillai's trace = 1.251, $F = 2.437$, df = 56, 300, $P < 0.001$). Individual ANOVAs conducted after MANOVA revealed significant differences between the experimental sites for each of these variables (Fig. 2).

Two components of the PCA performed with the abiotic matrix had eigenvalues greater than one, explaining over 67% of the total variance. The UTM coordinates and orientation showed the highest correlations with the first component of this PCA (Table 1). The second component of this PCA was most correlated with rainfall and slope.

Two components of the PCA performed with the composition–structure matrix had eigenvalues greater than one, explaining over 70% of the total variance. Perennial species diversity, the number of perennial plant patches and the area covered by sprouting shrubs were the variables showing the highest correlations with the first component of this PCA (Table 1). The cover and the spatial pattern of perennial vegetation were the variables most correlated to the second component. Again, two components of the PCA performed with the soil-surface matrix had eigenvalues greater than one, explaining over 89% of the total variance. The stability and nutrient cycling indices were strongly correlated with the first component of this PCA, and the infiltration index was strongly correlated to the second component (Table 1).

Results of the stepwise multiple regressions are shown in Table 2. The second component of the PCA conducted with the composition–structure matrix was the only variable selected in the case of $\delta^{13}C$ and the C:N ratio. The second component of the PCA conducted with the soil-surface matrix was the only variable selected in the case of $\delta^{15}N$. None of the components extracted from the abiotic, composition–structure and soil-surface matrices were selected as predictors of the foliar nitrogen concentration.

Discussion

Our hypothesis was supported by our results that ecosystem structural attributes and soil-surface conditions in the bare-ground areas were major determinants for the site-to-site differences in the foliar $\delta^{13}C$, $\delta^{15}N$ and C:N values of S. tenacissima in steppes from SE Spain. In these steppes, it has been demonstrated that water is redistributed from the bare-ground areas to the vegetated patches after rainfall events large enough to generate runoff, and that this water source is crucial for the growth of S. tenacissima (Puigdefábregas et al. 1999). Such redistribution of water typically occurs after rainfall events with more than 5 mm of rain and with intensities over 1.2 mm per 10 min (Puigdefábregas and Sánchez 1996; Cammeraat 2004). It is also favored by local differences in the infiltration rates between S. tenacissima tussocks and bare-ground areas, which are strongly related to the condition of the soil surface (Maestre et al. 2002), by the spatial pattern of this species (Cerdà 1997), and by other ecosystem structural attributes, such as the cover and size of S. tenacissima patches (Puigdefábregas et al. 1999). Our results complement previous studies (Pugnaire et al. 1996; Haase et al. 1999; Balaguer et al. 2002), and indicate that the structure of perennial vegetation and the condition of the soil surface in the bare-ground areas differentially affect the water and nutrient status of S. tenacissima.

It is interesting to point out the low site-to-site variation found in the average $\delta^{13}C$ values of S. tenacissima (1.2‰). Intraspecific variations in the foliar $\delta^{13}C$ values of less than 1‰ have been found across topographic gradients for Stipa capensis Thunb. (a grass), Rosmarinus officinalis L. (a shrub) and Juniperus thurifera L. (a tree) in a semiarid area of Spain (Peñuelas et al. 1999), and for Pinus massoniana Lamb. in China (Tateno et al. 2003). Similar intraspecific variations in the foliar $\delta^{13}C$ values of the Mediterranean

Fig. 2 Carbon and nitrogen isotopic composition ($\delta^{13}C$ and $\delta^{15}N$, respectively), nitrogen concentration and carbon-to-nitrogen ratio (C:N) of Stipa tenacissima leaves at the different study sites. Data represent means ± SE ($n=6$). The abbreviations in the x-axis correspond to the names of the experimental sites (see Fig. 1 for location). The experimental sites are ranked in ascending order of their index of aggregation (I_a) values. Different letters indicate significant differences between sites ($P < 0.05$, Tukey's b-test). Post hoc results for $\delta^{13}C$ and C:N data are shown for data transformed with power ($x^{4.5}$) and logarithmic functions, respectively
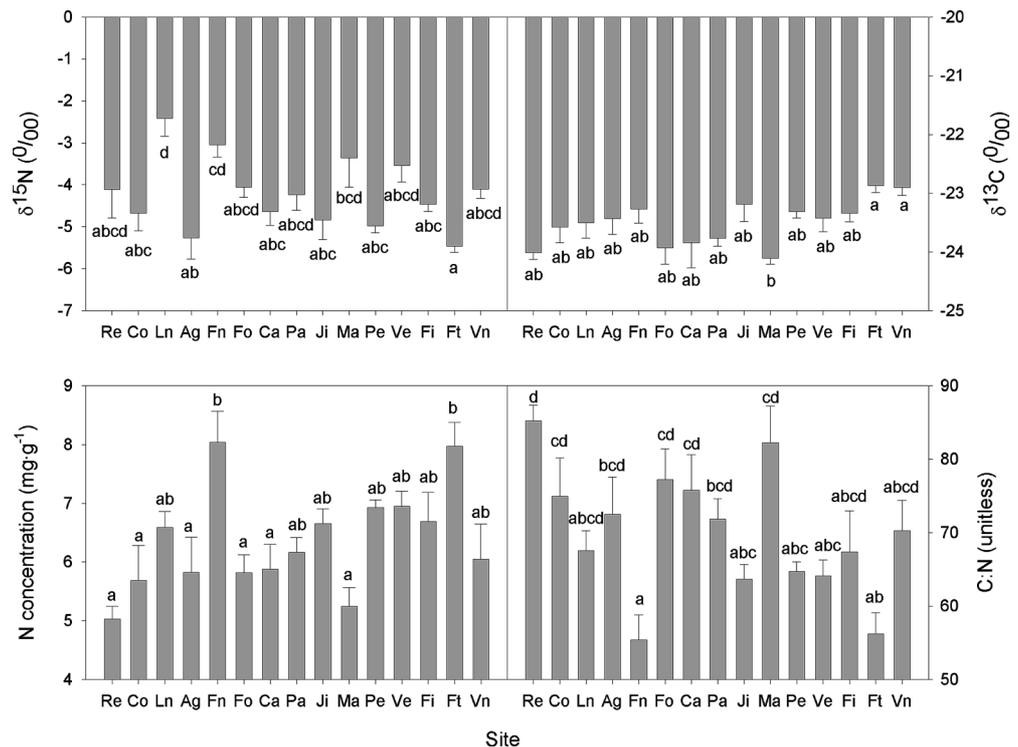
Table 1 Pearson correlations (*P* values) between the variables that constitute the different matrices and the PCA components which had eigenvalues greater than one

Matrix	Variable	PCA component	
		1	2
Abiotic	Mean annual rainfall	0.104 (0.7004)	0.915 (0.0001)
	Orientation	−0.681 (0.0044)	0.071 (0.7983)
	Slope	0.142 (0.6208)	−0.701 (0.0034)
	UTM coordinate east	0.857 (0.0001)	−0.265 (0.3406)
	UTM coordinate north	0.794 (0.0046)	0.266 (0.3161)
Composition–structure	Perennial species diversity	0.908 (0.0001)	0.043 (0.8804)
	Cover of perennial vegetation	0.335 (0.2316)	0.712 (0.0048)
	Area covered by sprouting shrubs	0.604 (0.0152)	0.307 (0.2710)
	Spatial pattern of perennial vegetation	0.015 (0.9556)	−0.887 (0.0001)
	Number of discrete perennial vegetation patches	0.886 (0.0001)	0.108 (0.7009)
Soil surface	Stability index	0.852 (<0.0001)	−0.378 (0.1650)
	Infiltration index	0.001 (0.9966)	0.959 (<0.0001)
	Nutrient cycling index	0.855 (<0.0001)	0.371 (0.1738)

In all cases, $n = 15$. The *P* values of the correlations involving variables from the abiotic and composition–structure matrices were obtained using a permutation test with 10,000 randomizations

Table 2 Multiple regression models fitted to the carbon (C) and nitrogen (N) isotopic composition ($\delta^{13}C$ and $\delta^{15}N$, respectively) and C:N ratio of *Stipa tenacissima* leaves

Variable (*Y*)	Model	R^2	ANOVA		
			F	df	P
$\delta^{13}C$ (‰)	$Y = -23.50 - 0.28 C2B$	0.54	15.25	1, 13	0.002
$\delta^{15}N$ (‰)	$Y = -4.21 - 0.48 C2S$	0.33	6.28	1, 13	0.026
C:N (unitless)	$Y = 69.93 + 4.72 C2B$	0.31	5.71	1, 13	0.033

C2B Second component of the PCA conducted with the composition–structure matrix, *C2S* second component of the PCA conducted with the soil-surface matrix. None of the PCA components was selected during the regression fitted to the nitrogen concentration

shrubs *E. multiflora* L., *Phyllirea latifolia* L. and *Globularia alypum* L. have been found in field experiments that manipulated water availability and microclimatic conditions (Llorens et al. 2003; Lloret et al. 2004). The low variation observed for *S. tenacissima* in our study is likely due to the site-to-site similarity in the dominant environmental factors affecting isotopic fractionation.

The second component of the PCA derived from the composition–structure matrix was the only significant predictor of the $\delta^{13}C$ values of *S. tenacissima*. According to the correlations between this component and the variables forming the composition–structure matrix, the total cover and the spatial pattern of perennial vegetation were the structural attributes that most influenced $\delta^{13}C$. These results suggest that an increase in total cover could promote a reduction in the foliar $\delta^{13}C$ of *S. tenacissima* and thus a reduction in the water use efficiency, and an increase in the spatial aggregation of perennial vegetation could promote an increase in the foliar $\delta^{13}C$ and thus promote an increase in the water-use efficiency(sensu Lajtha and Michener 1994). Increasing total vegetation cover can negatively affect the water use of *S. tenacissima* by modifying the amount of water carried away during runoff events and by increasing competition for water (Puigdefábregas et al. 1999).

At the catchment scale, it has been shown that the spatial pattern of vegetation in *S. tenacissima* steppes is determined by topography and the associated water fluxes (Puigdefábregas and Sánchez 1996). On moderate slopes, *S. tenacissima* tussocks tend to be aligned parallel to the contours; this maximizes their ability to trap and store water and sediments and gives the appearance of a regular spatial pattern (Puigdefábregas and Sánchez 1996). Our results provide a complementary view, and suggest that, at smaller spatial scales, an increase in the spatial aggregation of plant patches positively affects the water-use efficiency of *S. tenacissima*. It is interesting to point out the lack of significant differences between the sites in the values of the aggregation index (I_a; data not shown). This site-to-site similarity in the spatial pattern of perennial vegetation may contribute to explaining the low variability in the $\delta^{13}C$ values of *S. tenacissima* found among sites.

Despite the recognized importance of spatial patterns for ecosystem functioning and dynamics (Tilman and Kareiva 1997; Bolker et al. 2003), few studies have explicitly examined how the spatial structure of organisms per se may alter their performance (Murrell et al. 2001; Stoll and Prati 2001; Tirado and Pugnaire 2003). Albeit indirectly, our results suggest that the spatial pattern of vegetation per se may influence its own water use, and that within-site and between-site differences in I_a have different effects on the rainwater use of *S. tenacissima*. Previous studies have investigated how plant

spatial patterns affect plant water use and soil water balance in arid and semiarid ecosystems with a two-phase mosaic of vegetated patches and bare-ground soil (Slatyer 1961; see review by Tongway et al. 2001). However, we are not aware of any previous study evaluating how site-to-site changes in the spatial pattern of vegetation are related to the water use of the dominant species within a given ecosystem, as we did for *S. tenacissima*. The mechanisms underlying the relationships found cannot, however, be elucidated from our study, and merit further research.

The same component of the composition–structure matrix selected as predictor for $\delta^{13}C$ was selected as a predictor of the C:N values of *S. tenacissima* leaves. In this case, our results suggest that increasing cover would promote an increase in the C:N ratio and increasing spatial aggregation of perennial vegetation would promote a reduction in this ratio. Thus, these structural attributes of perennial vegetation affect the quality of *S. tenacissima* litter, as the C:N of plant tissues largely affects its decomposition once plant litter reaches the soil, affecting ecosystem processes such as soil respiration and N mineralization (Austin et al. 2004). As in the case of $\delta^{13}C$, our observational approach cannot provide a mechanistic interpretation of the relationships found. However, it provides empirical evidence of the potential importance that even subtle changes in ecosystem structure may have for ecosystem processes associated with plant performance.

The second component of the PCA derived from the soil-surface matrix was the only significant predictor of the $\delta^{15}N$ values of *S. tenacissima* tussocks. As this component was positively correlated with the infiltration index—a direct surrogate of the ability of the soil in the bare-ground areas to infiltrate rainfall—our results suggest that increasing infiltration in the between-patch bare-ground areas, or decreasing rainwater efflux to the vegetated patches may promote a reduction in the $\delta^{15}N$ values of *S. tenacissima*. In arid and semiarid ecosystems, N mineralization rates are largely determined by water availability, which is usually provided in the form of pulses after rainfalls (Austin et al. 2004). Roots of *S. tenacissima* do not exploit bare-ground areas (Puigdefábregas and Sánchez 1996), and thus the plant's nutrient- and water-uptake area is largely restricted to the area occupied by its canopy. In *S. tenacissima* steppes, as in other two-phase mosaic ecosystems, water inputs coming from runoff increase when infiltration in the bare-ground areas is low (Eldridge et al. 2000; Maestre et al. 2002). Thus, a reduction in the infiltration index in these areas would increase soil water availability underneath *S. tenacissima* tussocks which, in turn, would promote mineralization pulses and increase the amount of NO_3^- taken up by this species. As $\delta^{15}N$ of soil NO_3^- tends to be lower than that of soil NH_4^+ (Lajtha and Michener 1994), such an increase in NO_3^- uptake would reduce the foliar $\delta^{15}N$ of *S. tenacissima*, and thus provide some evidence for our results. However, it must be emphasized that the isotopic composition of the foliage is affected by multiple

causes not limited to mineralization, including the discrimination caused by processes such as nitrification and ammonification, the proportion of ^{15}N present in the soil exploited by the roots, the activity of mycorrhizae and other microorganisms, and the depth reached by the roots (Lajtha and Michener 1994; Handley et al. 1999; Dawson et al. 2002). Thus, the mechanisms underlying the relationships suggested by our results must be treated with caution, and should be confirmed by future studies.

Although we found a relatively wide range of variation in the nitrogen concentrations of *S. tenacissima* leaves, it was the only performance variable evaluated that did not show any significant relationship with the components extracted from the abiotic, composition–structure and soil-surface matrices. This result was unexpected, especially given the strong relationship existing between the average $\delta^{13}C$ and the nitrogen concentration of *S. tenacissima* leaves in the studied steppes (Appendix 2), and the importance that the variables sampled have for water and nutrient fluxes in arid and semiarid areas (Huxman et al. 2004). It also suggests that other factors not evaluated in this study having a key influence on N mineralization dynamics, such as the structure of microbial communities (Austin et al. 2004), could be important drivers of nitrogen uptake by *S. tenacissima*. We speculate with the possibility that differences between sites in the structure of these communities under the canopy of *S. tenacissima* could help to explain the variation in the nitrogen concentration observed in this study. Substantial differences between sites in the relative abundance of mycorrhizal propagules under the canopy of *S. tenacissima* have been found in the study area (Azcón-Aguilar et al. 2003).

Our results suggest that the relative importance of ecosystem structure and soil-surface conditions in the bare-ground areas was higher than that of abiotic factors as a determinant of the plant performance variables evaluated in this study. The lack of inclusion of the PCA components derived from the abiotic matrix as predictors in these regressions was noticeable, as variables like rainfall, slope and orientation largely determine soil water availability in arid and semiarid regions (e.g., Gómez-Plaza et al. 2001; Cantón et al. 2003). It also suggests that other key climatic features not included in our study, like the frequency and size of individual rainfall events, could be more relevant for the performance of *S. tenacissima* than the average annual rainfall (Reynolds et al. 2004). As the ability of this species to efficiently use rainwater pulses is likely to be influenced by the intensity, duration and frequency of individual rainfall events (Pugnaire et al. 1996; Haase et al. 1999), these variables could play an important role in the performance of *S. tenacissima*. However, such climatic variables are difficult to obtain in the study area, and their relative importance against the measured variables is unknown. Another factor not evaluated in this study that could also explain part of the site-to-site variation observed in the stable isotopic composition and nutrient

concentration of *S. tenacissima* is human disturbance. Although most human activities in the steppes of the study area due to the rapid industrialization of the country and the migration processes associated with it ceased in the 1960s (Barber et al. 1997), past human impacts are still influencing current perennial-vegetation richness and diversity at the study sites (Maestre 2004). Despite the fact that the influence of human disturbances on the isotopic composition of *S. tenacissima* will most likely be indirect—via effects on the structure and composition of the ecosystem—the role of these disturbances on the physiological performance of this species merits further research.

The results presented in this study add new empirical evidence showing the importance of ecosystem structure and soil-surface conditions in driving plant performance in arid and semiarid environments (e.g., García-Fayos et al. 2000; Maestre et al. 2003; Arbel et al. 2004). They also provide insights on the relative importance of different ecosystem attributes as drivers of plant performance in semiarid environments. Understanding how changes in ecosystem composition and structure affect plant performance is critical to accurately predicting the impacts of ongoing global change on ecosystems and communities (Simberloff 2004). Our results suggest that changes in ecosystem features such as plant spatial patterns and soil-surface conditions lead to modifications in the performance of *S. tenacissima*, and thus are likely to modify the dynamics of this species, and the ecosystem functions associated with it, in the mid- to long-term.

Appendix 1 Correlation matrix between the variables forming the composition–structure, abiotic and soil-surface matrices

	ELE	RAI	ORI	SLO	UTE	UTN	SPA	SRI	DIV	ASS	NPA	WPA	TCO	STA	INF
RAI	0.588														
ORI	0.260	0.034													
SLO	−0.031	−0.384	0.033												
UTE	−0.292	−0.218	−0.496	0.130											
UTN	0.595	0.367	−0.219	0.154	0.531										
SPA	−0.055	−0.217	0.049	0.604	0.032	−0.025									
SRI	0.866*	0.600	−0.062	−0.180	0.104	0.807*	−0.119								
DIV	0.491	0.449	0.075	−0.016	0.049	0.307	−0.129	0.450							
ASS	0.786*	0.643	0.018	−0.224	−0.222	0.513	−0.223	0.784*	0.458						
NPA	0.435	0.199	0.435	−0.243	−0.063	0.129	−0.099	0.409	0.734	0.355					
WPA	0.346	−0.215	0.173	−0.160	0.104	0.348	−0.372	0.385	0.069	0.157	0.277				
TCO	0.354	−0.135	0.160	−0.165	0.183	0.372	−0.371	0.440	0.239	0.276	0.416	0.915*			
STA	0.363	0.178	0.226	−0.610	−0.146	0.106	−0.573	0.422	0.270	0.490	0.557	0.581	0.661		
INF	0.591	0.402	0.067	0.256	−0.120	0.412	0.433	0.558	0.274	0.287	0.121	0.053	−0.033	−0.259	
NUT	0.531	0.439	−0.223	−0.452	0.237	0.490	−0.441	0.734	0.520	0.544	0.444	0.363	0.514	0.457	0.254

As some of the variables were non-normally distributed (Kolmogorov-Smirnoff test, $P < 0.05$), significance of the correlation coefficient was determined using a permutation test (10,000 randomizations) with the program Corr_permute (Pierre Legendre, Université de Montréal; downloaded from http://www.bio.umontreal.ca/casgrain/en/labo/corr_permute.html). Values marked by *asterisks* are significant ($P < 0.0029$) after adjusting for multiple testing using the false discovery rate (FDR) method of Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 57:289–300. For all variables, $n = 15$
ELE Elevation (m above sea level), *RAI* mean annual rainfall (in mm), *ORI* orientation (°), *SLO* slope (°), *UTE* UTM east coordinate, *UTN* UTM north coordinate, *SPA* Spatial pattern (SADIE's I_a, unitless), *SRI* species richness (n), *DIV* species diversity (Shannon index), *ASS* area covered by sprouting shrubs (m^2), *NPA* number of patches (number/10 m of linear transect), *WPA* width of patches (m/10 m of linear transect), *TCO* total cover of patches (%), *STA* LFA stability index (%), *INF* LFA infiltration index (%), *NUT* nutrient cycling index (%)
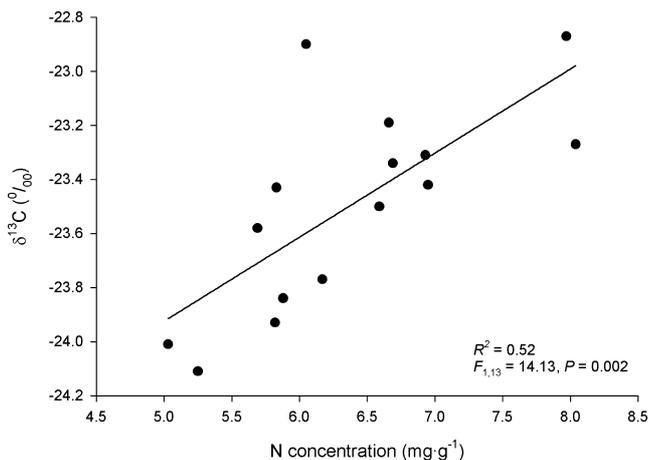
Appendix 2 Relationship between the average $\delta^{13}C$ and the average nitrogen concentration of *Stipa tenacissima* leaves in the studied steppes

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