Published in final edited form as:

Ecosystems. 2013 November ; 16(7): 1248–1261. doi:10.1007/s10021-013-9683-y.

On the importance of shrub encroachment by sprouters, climate, species richness and anthropic factors for ecosystem multifunctionality in semi-arid Mediterranean ecosystems

José L. Quero^{1,2,a,*}, Fernando T. Maestre^{1,b}, Victoria Ochoa^{1,c}, Miguel García-Gómez^{1,d}, and Manuel Delgado-Baquerizo³

¹Área de Biodiversidad y Conservación, Departamento de Biología y Geología, Escuela Superior de Ciencias Experimentales y Tecnología, Universidad Rey Juan Carlos, c/Tulipán s/n, 28933 Móstoles, Spain

²Departamento de Ingeniería Forestal, Campus de Rabanales Universidad de Córdoba, Carretera N-km. 396, 14071 Córdoba, Spain

³Departamento de Sistemas Físicos, Químicos y Naturales, Universidad Pablo de Olavide, Carretera de Utrera km. 1, 41013 Sevilla, Spain

Abstract

One of the most important changes taking place in drylands worldwide is the increase of the cover and dominance of shrubs in areas formerly devoid of them (shrub encroachment). A large body of research has evaluated the causes and consequences of shrub encroachment for both ecosystem structure and functioning. However, there are virtually no studies evaluating how shrub encroachment affects the ability of ecosystems to maintain multiple functions and services simultaneously (multifunctionality). We aimed to do so by gathering data from ten ecosystem functions linked to the maintenance of primary production and nutrient cycling and storage (organic C, activity of β -glucosidase, pentoses, hexoses, total N, total available N, amino acids, proteins, available inorganic P and phosphatase activity), and summarizing them in a multifunctionality index (M). We assessed how climate, species richness, anthropic factors (distance to the nearest town, sandy and asphalted road, and human population in the nearest town at several historical periods) and encroachment by sprouting shrubs impacted both the functions in isolation and M along a regional (ca. 350 km) gradient in Mediterranean grasslands and shrublands dominated by a non-sprouting shrub. Values of M were higher in those grasslands and shrublands containing sprouting shrubs (43% and 62%, respectively). A similar response was found when analyzing the different functions in isolation, as encroachment by sprouting shrubs increased functions by 2%–80% compared to unencroached areas. Encroachment was the main driver of changes in M along the regional gradient evaluated, followed by anthropic factors and

*Corresponding author, jose.quero@uco.es; phone: +34 957 218 385; fax: +34 957 212 095.

^afernando.maestre@urjc.es

^bvictoria.ochoa@urjc.es

^cmiguel.garciag@upm.es

^dmdelbaq@upo.es

Author contributions:

FTM and JLQ conceived the study; JLQ, FTM, VO, MGG and MDB performed research; JLQ and MDB analyzed data; and JLQ and FTM wrote the paper.

species richness. Climate had little effects on M in comparison to the other factors studied. Similar responses were observed when evaluating the functions in isolation. Overall, our results showed that M was higher at sites with higher sprouting shrub cover, longer distance to roads and higher perennial plant species richness. Our study is the first documenting that ecosystem multifunctionality in shrublands is enhanced by encroaching shrubs differing in size and leaf attributes. Our findings reinforce the idea that encroachment effects on ecosystem functioning cannot be generalized, and that are largely dependent on the traits of the encroaching shrub relative to those of the species being replaced.

Keywords

shrub invasion; ecosystem structure; ecosystem functioning; biodiversity; drylands; *Quercus coccifera*; *Rosmarinus officinalis*; *Stipa tenacissima*

Introduction

Drylands, regions with an aridity index (precipitation/potential evapotranspiration) value lower than 0.65 (UNCCD 1992), cover over 40% of the Earth's surface, provide key ecosystem services to more than 38% of the global human population (MEA 2005), and include highly diverse biomes that host about 20% of the major centers of plant diversity worldwide (White and Naeem 2003; Kier and others 2005). One of the most important changes taking place in these regions over the last 150 years is shrub encroachment, i.e. the increase of the cover and dominance of shrubs in areas formerly devoid of them, such as grasslands (van Auken 2009; Naito and Cairns 2011). Numerous papers have dealt with the causes and consequences of shrub encroachment for both ecosystem structure and functioning (see van Auken 2009 and Eldridge and others 2011 for recent reviews). The ecological consequences of shrub encroachment are not universal (Eldridge and others 2011), and the effects of this phenomenon on ecosystems range from negative to positive, depending on the region considered, the ecosystem function/s of interest and the organisms studied (Cable and others 2009; Jackson and others 2002; Maestre and others 2009; Ratajczak and others 2012).

The differences found in the literature between regions and ecosystem types regarding the ecological consequences of shrub encroachment may be due to the attributes of encroachers. Maestre and others (2009) hypothesized that the effects of shrub encroachment on ecosystem functioning are partially due to a set of key traits of potential encroachers relative to that of the grasses they replace. Eldridge and others (2011) tested this prediction indirectly, by using data available in the literature, and found support to these predictions, as traits such as the height and shape of the shrubs affected the structural and functional outcomes of their encroachment. However, and to the best of our knowledge, no previous study has directly evaluated in the field how the nature of encroaching shrubs relative to those of the species being replaced modulate their effects on ecosystem functioning. This kind of studies may shed some light on the variable ecological effects of encroaching shrubs found in literature.

Most of previous research evaluating the effects of shrub encroachment on ecosystem functioning has focused on evaluating only one or a few ecosystem functions at a time, such as productivity (Huenneke and others 2002), soil carbon and nitrogen contents (Jackson and others 2002; Wheeler and others 2007; Alberti and others 2011), and enzyme activities (Maestre and others 2011), to name a few. Although studies evaluating single variables are relevant, ecosystems are valued primarily for the multiple functions and services they provide (MEA, 2005), and shrub encroachment can affect multiple structural and functional variables at the same time (Eldridge and others 2011). Therefore, assessing how this phenomenon affects multiple ecosystem functions and services simultaneously, i.e., multifunctionality (Reiss and others 2009; Zavaleta and others 2010; Cardinale and others 2011), can provide greater insights on the ecological consequences of shrub encroachment, and help managers and policy makers to establish sound management actions. While the evaluation of multifunctionality is becoming prevalent in areas of ecology such as biodiversity–function research (Hooper and Vitousek 1998; Hector and Bagchi 2007; Gamfeldt and others 2008; Maestre and others 2012a), only Eldridge and others (2011) have attempted to explore the effects of shrub encroachment on multifunctionality so far, and their study was conducted with data from published studies that primarily focused on particular structural and functional attributes. Therefore, there is a critical need for field studies evaluating the effects of shrub encroachment on multifunctionality.

Being very important, shrub encroachment is not the only factor affecting ecosystem functioning in drylands. Previous studies have found that abiotic factors such as temperature and precipitation, and biotic factors such as species richness, can modulate ecosystem functioning along regional or global gradients (Paruelo and others 2004; Wang and others 2011; Maestre and others 2012a). Anthropogenic factors, such as past human activities, have been traditionally overlooked when studying drivers of ecosystem functioning in drylands. These have been found to be a major driver of ecosystem structure in different ecosystems (Waide and others 1999; Maestre 2004), and their effects on ecosystem functions could be even stronger, given that impacts of human activities on nutrient cycling and pools may last from decades to millennia (Dupouey and others 2002; Kacalek and others 2011; De Schrijver and others 2012). Studying anthropic factors is particularly important in areas with a long history of human use, such as the Mediterranean Basin (Blondel 2006), and when evaluating the functional consequences of shrub encroachment, as this phenomenon is largely driven by human-driven factors such as the introduction of grazers (di Castri 1981; Seifan and Kadmon 2006), changes in the regime of wildfires (Pausas 2004), and climate change (Archer and others 1995).

We evaluated how climate, species richness, anthropic factors and shrub encroachment by sprouting shrubs impacted ecosystem multifunctionality along a regional environmental gradient in semi-arid Mediterranean grasslands and shrublands. These landscapes are ideal ecosystems to do so, as both past human uses and shrub encroachment have been previously found to affect their structure and functioning (Maestre 2004; Maestre and others 2009). Mediterranean drylands are also suitable to test how the traits of the encroacher relative to those of the species being replaced affect the functional outcome of encroachment, since *Quercus coccifera* L., a large sprouting shrub is becoming more abundant at both grasslands dominated by the perennial grass *Stipa tenacissima* L. and shrublands dominated by the

obligate-seeder *Rosmarinum officinalis* L. (Supplementary Figure S1). Our first objective was to evaluate how increases in the cover and density of *Q. coccifera* affected multiple ecosystem functions (organic C, activity of β -glucosidase, pentoses, hexoses, total N, total available N, amino acids, proteins, available inorganic P and phosphatase activity), both in isolation and when evaluated simultaneously (i.e. multifunctionality), in both *S. tenacissima* grasslands and *R. officinalis* shrublands. We hypothesized that the magnitude of changes on ecosystem functions and multifunctionality with sprouting shrubs will be higher in grasslands than shrublands. We expect so because sprouting shrub leaves have lower C:N ratios than those of *S. tenacissima* (García and others 2005), and these shrubs can promote the activity and abundance of bacteria and fungi compared to grasses (Azcón-Aguilar and others 2003; Maestre and others 2009). Furthermore, they have higher biomass, primary production and litter productivity than *S. tenacissima* (Cañellas and Miguel 1998, 2000; Maestre and others 2007), and shrubs like *R. officinalis* have increased litterfall compared to *S. tenacissima* (Bochet and others 1998). Overall, these changes may lead to higher nutrient contents and improved physical soil properties under shrub canopies than under grass canopies (Bochet and others 1999; Maestre and Cortina 2004; Castillo-Monroy and others 2010; Supplementary Figure S2), maximizing the effects of encroachment by sprouting shrubs in *S. tenacissima* grasslands compared to *R. officinalis* shrublands. Our second objective was to evaluate the relative importance of sprouting shrubs, climate, anthropic effects and plant species richness on the ecosystem functions evaluated and on multifunctionality. We thus postulate that higher aridity and species richness will decrease and increase multifunctionality, respectively (Miranda and others 2009; Maestre and others 2012a), and that anthropic effects will reduce multifunctionality. We expect so because of the negative impacts that traditional and current human activities such as grazing, wood and fiber harvesting have on plant cover and species richness (Maestre 2004; Seifan and Kadmon 2006), and on processes such as erosion, nutrient cycling and storage (Tongway and others 2003; Bastida and others 2006).

Materials & Methods

Study area and experimental design

We surveyed 44 sites located along an environmental gradient from central to SE Spain (Supplementary Figure S3). Their climate is semi-arid, with annual rainfall and temperature varying from 339 mm to 479 mm, and from 12.4°C to 16.5°C, respectively (Supplementary Table S1). All plots were located on *Lithic Calciorthid* (Soil Survey Staff 1994) soils. This study was conducted in either grasslands or shrublands dominated by *S. tenacissima* and *R. officinalis*, respectively. Within grasslands (GR) and shrublands (SH), we selected sites with and without sprouting shrubs (SP), having then four types of communities, hereafter named as GR, GRSP, SH and SHSP (n = 11 sites per group). The dominant sprouting shrub was *Q. coccifera* in all cases, except in a SH site, where the sprouters were young trees of *Quercus ilex* L., a species highly related to *Q. coccifera* (Pearse and Hipp 2009). There were no differences among the four vegetation types studied regarding abiotic factors such as aridity index and elevation (Two-way ANOVA, $F_{3,40} < 1.23$, $P > 0.31$ in all cases). Total cover ranged from 36% to 68% in grasslands, and from 15% to 64% in shrublands. Sprouting shrub cover did not differ between GRSP (means $\pm$ s.e., $14 \pm 3\%$) and SHSP ($20\% \pm 4\%$)

sites (Two-way ANOVA, $F_{1,20} = 1.66$, $P = 0.21$). Thus, we had similar environmental conditions within both types of landscape (i.e., grasslands and shrublands) along the climatic gradient, and in both of them the sites with sprouting shrubs had similar cover values.

Composition and structure of vascular plants: biotic factors

At each site, we established a 30 m $\times$ 30 m plot in an area representative of its overall vegetation. We assessed the cover of perennial vegetation using the line-intercept method in four 30-m long transects per plot, which were extended parallel to the slope and located 8 m apart. In each transect, we also placed 20 consecutive quadrats (1.5 m $\times$ 1.5 m size), where the cover of each perennial species was visually estimated. We restricted our study to perennial plants because they are instrumental in maintaining ecosystem functioning and preventing desertification in drylands (Whitford 2002, Maestre and Escudero 2009). The total number of perennial species found in the 80 quadrats was used as our surrogate for species richness per plot. With this information, we calculated an encroachment index per plot, defined as $SPC - nSPC / TC$, being SPC the cover of sprouting shrubs, nSPC the cover of non-sprouting shrubs and TC the total vegetation cover. This index provides a numerical and continuous indicator of sprouting shrub cover, taking into account the cover of non-sprouting shrubs and the total cover per plot, in order to control potential effect of both factors on our response variables across plots.

Abiotic, climatic, geographic and anthropic variables

The coordinates of each plot were recorded *in situ* with a portable Global Positioning System (GPS), and were standardized to the WGS84 ellipsoid for visualization and analyses. GPS coordinates were used to obtain 21 standardized climatic variables from Worldclim (www.worldclim.org), a high resolution (30 arc seconds or ~1km at equator) climate database (Hijmans and others 2005). We then conducted a principal component analysis (PCA) using a correlation matrix with the 21 climatic variables (Supplementary Table S2). The three first components of this PCA (hereafter named *Clim-Ax1*, *Clim-Ax2*, *Clim-Ax3*) had eigenvalues higher than one, and thus were retained for further analyses (Quinn and Keough 2002); these axes explained 92.5% of variation in climatic data. *Clim-Ax1* was negatively related to isothermality [i.e., (Mean Diurnal Range / Temperature Annual Range) ($\times 100$)], *Clim-Ax2* was positively and negatively related to annual precipitation and annual mean temperature, respectively, while *Clim-Ax3* was positively related to the average diurnal range in temperature [i.e., Mean of monthly (max temp - min temp)] (Supplementary Table S2). The slope angle of each plot was measured *in situ* with a clinometer.

We used the following variables as surrogates of human impacts at the experimental sites: distance to the nearest town, human population in the nearest town at several historical periods (1857, 1887, and 1950), distance to the nearest dirt road and distance to the nearest asphalted road. The Euclidean distance between the center of each plot and the center of the nearest town/city and asphalted/dirt road was obtained with Google Earth (www.google.com/earth/index.html). We assumed that the closer a given plot to the nearest city/town and dirt/asphalted road, the more likely that site to have suffered from greater human impacts in the past (Calef and others 2008; Rogala and others 2011). Population dates were selected because they represent the first census per municipality available in

Spain (1857), and two moments of special human pressure on natural resources because of the lack of woody fuel (1887) and the socio-economic situation following the Spanish Civil War (1950; Servicio del Esparto, 1950; López et al. 1992). Population data were obtained from official statistics (www.ine.es). We conducted a PCA using a correlation matrix with the different human variables selected. The three first components of this PCA (hereafter named *Anth-Ax1*, *Anth-Ax2*, *Anth-Ax3*) had eigenvalues higher than one, and thus were retained for further analyses (Quinn and Keough 2002); these axes explained ~79% of variation in anthropic data. *Anth-Ax1* was positively correlated to human population in the three dates (Pearson's $r > 0.70$ for the three variables), *Anth-Ax2* was positively correlated to distance to the nearest asphalted road (Pearson's $r = 0.90$) and *Anth-Ax3* was positively correlated to distance to the nearest dirt road (Pearson's $r = 0.98$).

Assessment of ecosystem multifunctionality

Soil sampling and laboratory analyses were conducted as described in Supplementary Information S1. We evaluated ten soil variables (ecosystem functions hereafter) linked to the maintenance of primary production and nutrient cycling and storage (organic C, activity of β -glucosidase, pentoses, hexoses, total N, total available N, amino acids, proteins, available inorganic P and phosphatase activity; see Supplementary Information S1 for a rationale of the variables employed). Although we also analyzed all these ecosystem functions separately, they were summarized in a multifunctionality index (*M*), calculated as the average of the Z scores of the ten ecosystem functions estimated at the plot level (Maestre and others 2012a; Supplementary Information S1). This index provides a straightforward and easily interpretable measure of multifunctionality. We acknowledge that, by using such an average, declines in one function can theoretically be compensated for by increases in one or another function (Gamfeldt and others 2008; 2013). However, we did not find that sites with high values of a single or a few functions had consistently low values for other functions. Indeed, the correlations between our ecosystem functions were either positive or close to 0 in most cases (Supplementary Table S3). Therefore, the higher the *M* values, the higher the multifunctionality. Similarly, we also obtained functions from the N, C, and P cycles by averaging the Z scores of the variables involved in each cycle, which were named as *N*, *C*, and *P* indices, respectively. Indices such as those used here are being increasingly used in the multifunctionality literature (e.g., Zavaleta and others 2010; Mouillot and others 2011; Freudenberger and others 2012; Maestre and others 2012b; Bowker and others 2013), as they summarize and integrate the effects of multiple functions into a single metric that is easily interpretable and analyzable quantitatively. The multifunctionality indices employed here also have good statistical properties, as they follow a normal distribution (Kolmogorov-Smirnov tests, $P > 0.2$ for the four cases), and are highly related to other multifunctionality indices proposed in the literature (Supplementary Figure S4).

Statistical analyses

We first explored the effects of sprouting shrubs on the functions evaluated and on multifunctionality in both grasslands and shrublands by using two-way ANOVA, with landscape type (L, grassland vs. shrubland) and the presence/absence of sprouting shrubs (R) as main, fixed, factors. Results revealed a lack of interactions between both factors for *M*, and for *C*, *N* and *P* indices, as well as for every functions evaluated but total available N,

suggesting that encroachment had the same effects on shrublands and grasslands (Figures 1 and 2; Supplementary Tables S4 and S5). Given these results, we pooled data from both vegetation types for further analyses.

We followed a multi-model inference approach (Burnham and Anderson 2002), which uses information theory to assess the probability that a given model is the most appropriate description of the observed data (Johnson and Omland, 2004). We evaluated all possible linear regression models containing the different functions evaluated, *M*, *C*, *N* or *P* index as the dependent variable and the following independent variables: encroachment index, species richness, slope, climatic and anthropogenic factors. Due to the limited number of sites that we have in this study ($n=44$), we had to keep a unique climatic and anthropogenic PCA component to avoid problems related to overfitting and spurious effects (Burnham and Anderson 2002). Thus, we used the *Clim-Ax1* and *Anthr-Ax3* in our models, as they were the components most related to the *M*, *C*, *N* and *P* indices, and to most of the functions evaluated (Supplementary Table S6). The variance inflation factor between the independent variables included in our models was below four, suggesting the absence of collinearity problems (Chatterjee and Price 2001). Model selection criterion is described in Supplementary Information S2.

The characteristics of our survey make the presence of spatial autocorrelation likely, which may be a problem in multi-model approaches based on information theory (Hoeting and others 2006). To check the potential effects of spatial autocorrelation in the data, a Moran's I correlogram was done on the residuals of either ANOVAs or the best model from the multi-model analysis. ANOVA analysis was carried out with Statistica v7 software and correlograms and multi-model analyses were carried out with the SAM 4.0 software (Rangel et al. 2010). The data used in this article are deposited in the Dryad repository: <http://dx.doi.org/>.

Results

The presence of sprouting shrubs increased *M*, *C*, *N* and *P* indices (Figure 1, Supplementary Table S4). This trend was followed for most of the ecosystem functions when analyzed separately (Fig. 2, Supplementary Table S5). Moran's I correlograms of residuals from ANOVA analyses showed a lack of spatial autocorrelation in the different response variables evaluated, suggesting that our results were not biased by spatial autocorrelation (Supplementary Figure S5).

When considering predictor variables other than sprouting shrubs, results showed that the best (smallest AIC_c) and most parsimonious (fewest variables with AIC_c values comparable to those of the best model, i.e., $AIC_c < 2$) models describing the multifunctionality index (*M*) contained three and two predictor variables, respectively (Table 1). Both models explained around 40% of the variance found in *M*, and included the encroachment index and the third anthropic PCA component (*Ant-Ax3*) as predictor variables. The best model also included plant species richness. On the contrary, the first climatic PCA component (*Clim-Ax1*) was included neither the best nor the most parsimonious models explaining *M*, *C*, *N*, and *P* indices. Slope was also included in the best model explaining the *P* index. Residuals

of the best four models did not suffer from spatial autocorrelation, as indicated by Moran's I correlograms (Supplementary Figure S6). Similar results were found when ecosystem functions were separately analyzed (Supplementary Table S7). Variables such as organic carbon, activity of β -glucosidase, total nitrogen or amino acid content included the encroachment index, plant species richness and the third anthropic PCA component (*Anth-Ax3*) as predictor variables in their most important models. Variables related to P cycle such as available P similarly include slope in the best model explaining this variable. The encroachment index, the *Anth-Ax3* component and species richness were the most important predictors of *M* (Figure 3). The three independent variables were positively related to *M*, indicating that higher ecosystem functionality was found at those sites with higher cover of sprouting shrubs, longer distance to roads and higher species richness (Table 2). Additionally, the *Clim-Ax1* component and slope had a low relative importance as predictors of *M* (Figure 3). The removal of *Anth-Ax3* as a predictor variable substantially reduced the fit of the model (Table 1). Thus, for the *M* index, the first and the second models of Table 1 are the best and the most parsimonious models, respectively; the same models without the *Anth-Ax3* component had $R^2 = 0.368$, $AICc = 70.95$, $AICc = 7.52$; and $R^2 = 0.276$, $AICc = 74.53$, $AICc = 5.09$, respectively.

Similar results were found when functions related to the C and N cycles (i.e., *C* and *N* indices) were evaluated separately (Table 1). The encroachment index, the *Anth-Ax3* component and species richness were the most important predictors of *C* and *N* indices. In both cases, the relative importance of the *Clim-Ax1* component and slope was low (Figure 3). The encroachment index, the *Anth-Ax3* component and species richness were positively related to *C* and *N* indices, meaning that higher ecosystem functionality related to C and N cycles was found at those sites with higher cover of sprouting shrubs, longer distance to roads, and higher species richness (Supplementary Table S8). For the *C* index, the first model of Table 1 are the best and the most parsimonious model, and did not include the *Anth-Ax3* component. For the *N* index, the first and the second models of the table are the best and the most parsimonious models, respectively. For this index, the removal of the *Anth-Ax3* component only reduced the model fit in the second case ($R^2 = 0.230$, $AICc = 87.024$, $AICc = 5.11$).

The encroachment index, the *Anth-Ax3* component and slope were the most important predictors of the *P* index (Figure 3). The relative importance of the *Clim-Ax1* component and species richness was low (Figure 3). The encroachment index and the *Anth-Ax3* component were positively related to *P* index, and slope was negatively related to this index, meaning that higher ecosystem functionality related to P cycle was found at those sites with higher cover of sprouting shrubs, longer distance to roads and smoother sites (Supplementary Table S8). The fit of the models adjusted to the *P* index was substantially reduced when removing the *Anth-Ax3* component as a predictor variable. The first and the second models of Table 1 are the best and the most parsimonious models, respectively for the *P* index; the same models without the *Anth-Ax3* component had $R^2 = 0.257$, $AICc = 102.59$, $AICc = 2.66$; and $R^2 = 0.213$, $AICc = 102.70$, $AICc = 2.15$, respectively.

Similarly to M, C, N and P indices, functions analyzed separately showed similar results. The encroachment index was one of the most important predictor for all ecosystem functions

but protein content. Species richness was also an important predictor for hexose, amino acid and protein contents, and the *Anth-Ax3* component was important for organic carbon, total nitrogen, total available nitrogen and phosphatase extracellular activity. Slope was an important predictor for available phosphorus (Figure 4).

Discussion

Given the extent of shrub encroachment worldwide, it is not surprising to find an increasing number of studies focused on the ecological consequences of this phenomenon (Supplementary Figure S7). However, the literature is still dominated by studies focusing on single functional variables, such as soil C or aboveground net primary productivity. To our knowledge, our study is the first attempt specifically designed to directly evaluate in the field the effects and relative importance of shrub encroachment as a driver of ecosystem multifunctionality. In addition, two different vegetation types (i.e., grasslands and shrublands) along a regional gradient have been studied, since the encroachment of sprouting shrubs has been observed not only in *S. tenacissima* grasslands, but also in shrublands dominated by the non-sprouter *R. officinalis* (Supplementary Figure S1). Given that shrub encroachment in previously-established shrublands has also been reported in other dryland regions (Noble 1997, Daryanto and Eldridge 2010), our approach allows extending the generality of the potential effects of shrub encroachment in landscapes other than grasslands. Overall, our results showed that sprouting shrub encroachment, plant species richness and anthropic factors made important contributions to maintain ecosystem multifunctionality, being this variable higher in both grasslands and shrublands where sprouting shrubs appeared.

Contrary to our first hypothesis, we did not find an interaction between vegetation type and the presence/absence of sprouting shrubs. The magnitude of the functional changes promoted by the encroachment of sprouting shrubs was similar in both grasslands and shrublands dominated by an obligate-seeder shrub. Maestre and others (2009) hypothesised that one of the variables involved in the changes on ecosystem functioning promoted by shrub encroachment is the suite of traits of the encroaching shrub in comparison to that the grasses being replaced. Our results extend this hypothesis to other shrubs that, similarly to grasses, are not functionally equivalents to the encroachers. *Quercus coccifera* is typically taller than *R. officinalis* and *S. tenacissima* (Blanca and others 2011). Larger shrubs provide more shade, reducing soil temperature, and subsequently increasing soil moisture (Maestre and others 2003). In this direction, Eldridge and others (2011) found a positive relationship between the height of the encroachers and improvements in ecosystem functioning. Similarly to height, *Q. coccifera* has a sprawling canopy and higher basal area than *R. officinalis* and *S. tenacissima* and, consequently, higher capacity of producing litter and higher retention of runoff and sediments (Cañellas and Miguel 1998). Thus, higher litter/debris mass and resource capture by *Q. coccifera* would be likely to promote higher levels of microbial decomposition, leading to enhanced C and N pools (McClaran and others 2008; Throop and Archer 2008). This reasoning agrees with the observed increment in ecosystems functions related to these pools such as organic C and amino acid contents to name a few (Figure 2). Moreover, leaves of *Q. coccifera* have higher N, C, and protein contents than *R. officinalis*, while the latter has higher caloric contents, construction costs, ashes and lipids

than *Q. coccifera* (Villar and Merino, 2001). On the other hand, *S. tenacissima* leaves have a high fiber content (Bessadok and others 2007; Belkhir and others 2012), and typically show lower N contents than *Q. coccifera* leaves (Maestre and Cortina 2006). *Stipa tenacissima* and *R. officinalis* have higher foliar C:N ratios than *Q. coccifera* (Ferrán and others 2005, Maestre and Cortina 2006), and this may lead to higher decomposition rates (Zhang and others 2008) of the litter of *Q. coccifera*. Thus, the differences in important functional traits between *Q. coccifera* and both *R. officinalis* and *S. tenacissima* may underlie the enhancement in ecosystem multifunctionality observed after the encroachment of grasslands and shrublands by *Q. coccifera*.

Results of the model inference approach showed that species richness and the third anthropic PCA component (*Ant-Ax3*) also explained an important part of the variation found in the *M*, *C*, *N* and *P* indices, as well as in most ecosystem functions, as we stated in our second hypothesis. Regarding plant species richness, the relationship between this predictor and these indices was positive in all cases. It has been suggested that biodiversity may be one of the major drives of ecosystem changes in the 21st century (Hooper and others 2012), and results reported here support this statement given the positive change on ecosystem multifunctionality observed with increases in species richness. These results are similar to those found in Maestre and others (2012a), where multifunctionality was positively related to species richness in global drylands comprising multiple vegetation types. In our study sites, one could expect that this positive richness-function relationship is mediated by nucleation processes mediated by *Q. coccifera*, given the attraction that this species exert on birds that feed on its acorns and disperse other endozoochorous seeds when perched (Verdú and Garcia-Fayos 1996; Bonfil 1998; Gómez 2003). Alternatively, stronger facilitative effects of *Q. coccifera* compared to those of *R. officinalis* and *S. tenacissima* could be due to the better soil conditions provided by the former (i.e., higher available N in *Q. coccifera*; Supplementary Figure S2), which contribute to an increase of species richness at the entire community level (Soliveres and others 2011). However, the lack of correlation between species richness and the cover of sprouting shrubs (Pearson's, $r = 0.08$, $n = 44$, $P = 0.6$) may exclude nucleation processes as a potential explanation of the patterns observed. This lack of relationship between species richness and shrub cover contrasts with other studies showing negative (Selene and Collins 2008; Ratajczak and others 2012), positive (Cabral and others 2003) or negative and positive (Dickie and others 2011) relationships between both variables, and supports that the encroachment effects on diversity should not be generalized among different ecosystems.

In addition, our modelling approach also showed that anthropic factors had negative effects on multifunctionality (Table 2, Supplementary Table S6). The *Ant-Ax3* component was positively related to *M* index and distance to roads, meaning that higher ecosystem multifunctionality was found at sites where roads were more distant. These results followed our expectations, given the strong effects of past human uses on the Mediterranean Basin (Blondel 2006), and previous studies showing that ecosystem properties depend to a large extent not only on biotic, but also on anthropic effects (Hooper and others 2005; García and Martínez 2012). What is most surprising here is that, despite the variables selected here as proxies of human impacts were indirect measurements, they explained a significant part of the variation in multifunctionality. In fact the removal of the *Ant-Ax3* component as a

predictor variable substantially reduced the fit of the model adjusted to M (Table 1). More accurate data regarding fire frequencies, grazing and/or harvest intensities per plot would have been even better predictor of ecosystem functions, but these data were not available for our sites, as the consulted historical archives did not have the spatial resolution required for the approach followed here. Nevertheless, it is important to note the fact that direct, “on-site” measurements such as encroachment and plant species richness had similar importance than indirect measurements of human impacts. Such measurements, which are easy to obtain and – as shown by our results – informative, offer great promise to improve our knowledge on the factors affecting ecosystem structure and functioning, particularly in those ecosystems with a long history of human use.

Regarding climate, it has been shown that increases and decreases on temperature and precipitation, respectively, can reduce ecosystem multifunctionality in global drylands (Maestre and others 2012a). In our work, multifunctionality tracks these trends. However, the effect of climate was surprisingly less important than that of encroachment, species richness and anthropic factors. This is likely due to the relatively narrow gradient of precipitation and temperature existing among the studied sites (Supplementary Table S1). Effects of small-scale variables on ecosystem functions may be more important than large-scale climatic variation. Indeed, the level of encroachment and species richness, which are within-site variables more directly related to soil processes (García-Fayos and Bochet 2009), were the most important variables driving ecosystem multifunctionality.

In summary, we conclude that the encroachment by sprouting shrubs enhances ecosystem multifunctionality in semi-arid Mediterranean grasslands and shrublands, and that both shrub encroachment and species richness were the most important drivers of ecosystem functioning and multifunctionality along the environmental gradient studied. Our study is the first documenting that ecosystem multifunctionality in shrublands is enhanced by encroaching shrubs differing in size and leaf attributes. Our findings reinforce the idea that encroachment effects on ecosystem functioning cannot be generalized, and that are largely dependent on the traits of the encroaching shrub relative to those of the species being replaced.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

We thank B. Gozalo, E. Valencia, M. Berdugo, MD. Puche, A. Gallardo, C. Alelly and A. Prado for their help with the laboratory analyses, A. Puche, C. Escolar and J.G. Illán for their help with field surveys, and Ann Kinzing and two anonymous reviewers for their comments on previous versions of this manuscript. This research was funded by the European Research Council under the European Community’s Seventh Framework Programme (FP7/2007-2013)/ERC Grant agreement n° 242658 (BIOCOM) awarded to FTM. JLQ acknowledges support from the BIOCOM project. MDB was supported by the Ministry of Science and Innovation of the Spanish Government, Grant n° CGL2010-21381. FTM also acknowledges support from the Spanish Ministerio de Educación (“Salvador de Madariaga program”, PR2010-0230) during the writing of the ms.

References

- Alberti G, Leronni V, Piazzì M. Impact of woody encroachment on soil organic carbon and nitrogen in abandoned agricultural lands along a rainfall gradient in Italy. *Reg Environ Change*. 2011; 11:917–924.
- Archer SR, Schimel DS, Holland EH. Mechanisms of shrubland expansion: land use, climate or CO₂. *Climatic Change*. 1995; 29:91–99.
- Azcón-Aguilar C, Palenzuela J, Roldán A, Bautista S, Vallejo R, Barea JM. Analysis of the mycorrhizal potential in the rhizosphere of representative plant species from desertification-threatened Mediterranean shrublands. *Appl Soil Ecology*. 2003; 22:29–37.
- Bastida F, Moreno JL, Hernández T, García C. Microbiological activity in a soil 15 years after its revegetation. *Soil Biol Biochem*. 2006; 38:2503–2507.
- Belkhir S, Koubaa A, Khadhri A, Ksontini M, Smiti S. Variations in the morphological characteristics of *Stipa tenacissima* fiber: The case of Tunisia. *Ind Crop Prod*. 2012; 37:200–206.
- Bessadok A, Marais S, Gouanve F, Colasse L, Zimmerlin I, Roudesli S, Metayer M. Effect of chemical treatments of Alfa (*Stipa tenacissima*) fibres on water-sorption properties. *Compos Sci Technol*. 2007; 67:685–697.
- Blanca, G. Flora Vascular de Andalucía oriental. 2ª edición corregida y aumentada. Cabezudo, B.; Cueto, M.; Fernandez Lopez, C.; Morales Torres, C., editors. Consejería de medio Ambiente, Junta de Andalucía; Sevilla: 2011.
- Blondel A. The 'Design' of Mediterranean Landscapes: A Millennial Story of Humans and Ecological Systems during the Historic Period. *Hum Ecol*. 2006; 34:713–729.
- Bochet E, Rubio JL, Poesen J. Relative efficiency of three representative matorral species in reducing water erosion at the microscale in a semi-arid climate (Valencia, Spain). *Geomorphology*. 1998; 23:139–150.
- Bochet E, Rubio JL, Poesen J. Modified topsoil islands within patchy Mediterranean vegetation in SE Spain. *Catena*. 1999; 38:23–44.
- Bonfil C. The effects of seed size, cotyledon reserves and herbivory on seedling survival and growth in *Quercus rugosa* and *Quercus laurina* (Fagaceae). *Am J Botany*. 1998; 85:79–87. [PubMed: 21684882]
- Bowker, MA.; Maestre, FT.; Mau, RL. Diversity and Patch-Size Distributions of Biological Soil Crusts Regulate Dryland Ecosystem Multifunctionality. *Ecosystems* in press; 2013.
- Burnham, KP.; Anderson, DR. Model Selection and Multimodel Inference. A Practical Information-Theoretical Approach. Springer-Verlag; New York: 2002.
- Cable JM, Ogle K, Tyler A. Woody plant encroachment impacts on soil carbon and microbial processes: results from a hierarchical Bayesian analysis of soil incubation data. *Plant Soil*. 2009; 320:153–167.
- Cabral, AC.; De Miguel, JM.; Rescia, JAJ.; Schmitz, MF.; Pineda, FD. Shrub encroachment in Argentinean savannas. *J Veg Sci*. Vol. 14. Cambridge Univ. Press; Cambridge: 2003. p. 145–152.
- Calef MP, McGuire AD, Chapin FS III. Human influences on wildfire in Alaska from 1988 through 2005: an analysis of the spatial patterns of human impacts. *Earth Interact*. 2008; 12:1–17.
- Cañellas I, Miguel AS. Litter fall and nutrient turnover in Kermes oak (*Quercus coccifera* L.) shrublands in Valencia (eastern Spain). *Ann Sci Forest*. 1998; 55:589–597.
- Cañellas I, Miguel AS. Biomass of root and shoot systems of *Quercus coccifera* shrublands in Eastern Spain. *Ann For Sci*. 2000; 57:803–810.
- Cardinale BJ, Matulich KL, Hooper DU, Byrnes JE, Duffy E, Gamfeldt L, Balvanera P, O'Connor MI, Gonzalez A. The functional role of producer diversity in ecosystems. *Am J Bot*. 2011; 98:572–592. [PubMed: 21613148]
- Castillo-Monroy AP, Maestre FT, Delgado-Baquerizo M, Gallardo A. Biological soil crusts modulate nitrogen availability in semi-arid ecosystems: Insights from a Mediterranean grassland. *Plant Soil*. 2010; 333:21–34.
- Chatterjee, S.; Price, B. Regression Analysis by Example. ed. 2. Wiley; New York: 2001.

- Daryanto S, Eldridge DJ. Plant and soil surface responses to a combination of shrub removal and grazing in a shrub–encroached woodland. *J Environ Manage.* 2010; 91:2639–2648. [PubMed: 20696514]
- De Schrijver A, Vesterdal L, Hansen K, De Frenne P, Augusto L, Achat DL, Staelens J, Baeten L, De Keersmaecker L, De Neve S, Verheyen K. Four decades of post-agricultural forest development have caused major redistributions of soil phosphorus fractions. *Oecologia.* 2012; 169:221–234. [PubMed: 22120703]
- di Castri, F. Mediterranean-type shrublands of the world. *Mediterranean-type Shrublands.* Di Castri, F., et al., editors. Elsevier; Amsterdam: 1981. p. 1–52.
- Dickie IA, Yeates GW, St John MG, Stevenson BA, Scott JT, Rillig MC, Peltzer DA, Orwin KH, Kirschbaum M, Hunt JE, Burrows LE, et al. Ecosystem service and biodiversity trade-offs in two woody successions. *J Appl Ecol.* 2011; 48:926–934.
- Dupouey JL, Dambrine E, Laffite D, Moares C. Irreversible impact of past land use on forest soils and biodiversity. *Ecology.* 2002; 83:2978–2894.
- Eldridge DJ, Bowker MA, Maestre FT, Roger E, Reynolds JF, Whitford WG. Impacts of shrub encroachment on ecosystem structure and functioning: towards a global síntesis. *Ecol Lett.* 2011; 14:709–722. [PubMed: 21592276]
- Ferran A, Delitti W, Vallejo VR. Effects of fire recurrence in *Quercus coccifera* L. shrublands of the Valencia Region (Spain): II plant and soil nutrients. *Plant Ecol.* 2005; 177:71–83.
- Freudenberger L, Hobson PR, Schluck M, Ibisch PL. A global map of the functionality of terrestrial ecosystems. *Ecol Complex.* 2012; 12:13–22.
- Gamfeldt L, Hillebrand H, Jonsson PR. Multiple functions increase the importance of biodiversity for overall ecosystem functioning. *Ecology.* 2008; 89:1223–1231. [PubMed: 18543617]
- Gamfeldt L, Snäll T, Bagchi R, Jonsson M, Gustafsson L, Kjellander P, Ruiz-Jaen MC, Fröberg M, Stendahl J, Philipson CD, Mikusinski G, et al. Higher levels of multiple ecosystem services are found in forests with more tree species. *Nat Commun.* 4:1340. [PubMed: 23299890]
- García C, Roldán A, Hernández T. Ability of different plant species to promote microbiological processes in semiarid soil. *Geoderma.* 2005; 124:193–202.
- García D, Martínez D. Species richness matters for the quality of ecosystem services: a test using seed dispersal by frugivorous birds. *Proc R Soc Lond B.* 2012; 279:3106–3113.
- García-Fayos P, Bochet E. Indication of antagonistic interaction between climate change and erosion on plant species richness and soil properties in semiarid Mediterranean ecosystems. *Glob Change Biol.* 2009; 15:306–318.
- Gómez JM. Spatial patterns in long-distance dispersal of *Quercus ilex* acorns by jays in a heterogeneous landscape. *Ecography.* 2003; 26:573–584.
- Hector A, Bagchi R. Biodiversity and ecosystem multifunctionality. *Nature.* 2007; 448:188–190. [PubMed: 17625564]
- Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. Very high resolution interpolated climate surfaces for global land areas. *Int J Climatol.* 2005; 25:1965–1978.
- Hoeting JA, Davis RA, Merton AA, Thompson SE. Model selection for geostatistical models. *Ecol Appl.* 2006; 16:87–98. [PubMed: 16705963]
- Hooper DU, Carol E, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, Gonzalez A, Emmett J, Gamfeldt L, O'Connor M. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. *Nature.* 2012; 486:105–108. [PubMed: 22678289]
- Hooper DU, Chapin FS III, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, et al. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr.* 2005; 75:3–35.
- Hooper DU, Vitousek PM. Effects of plant composition and diversity on nutrient cycling. *Ecol Monogr.* 1998; 68:121–149.
- Huenneke LF, Anderson JP, Remmenga M, Schlesinger WH. Desertification alters patterns of aboveground net primary production in Chihuahuan ecosystems. *Glob Change Biol.* 2002; 8:247–264.
- Jackson RB, Banner JL, Jobbagy EG, Pockman WT, Wall DH. Ecosystem carbon loss with woody plant invasion of grasslands. *Nature.* 2002; 418:623–626. [PubMed: 12167857]

- Johnson JB, Omland KS. Model selection in ecology and evolution. *Trends Ecol Evol.* 2004; 19:101–108. [PubMed: 16701236]
- Kacalek D, Dusek D, Novak J, Slodicak M, Bartos J, Cernohous V, Balcar V. Former agriculture impacts on properties of Norway spruce forest floor and soil. *Forest Systems.* 2011; 20:437–443.
- Kier G, Mutke J, Dinerstein E, Ricketts TH, Küper W, Kreft H, Barthlott W. Global patterns of plant diversity and floristic knowledge. *J Biogeogr.* 2005; 32:1–10.
- López, G.; Rico, L.; Martin, C. Els vertebrats terrestres de la Comarca d'Alacant. Caja de Ahorros Provincial de Alicante; Alicante. Spain: 1992.
- Maestre FT, Bautista S, Cortina J. Positive, negative and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. *Ecology.* 2003; 84:3186–3197.
- Maestre FT, Bowker MA, Puche MD, Hinojosa MB, Martínez I, García-Palacios P, Castillo AP, Soliveres S, Luzuriaga AL, Sánchez AM, Carreira JA, et al. Shrub encroachment can reverse desertification in Mediterranean semiarid grasslands. *Ecol Lett.* 2009; 12:930–941. [PubMed: 19638041]
- Maestre FT, Castillo-Monroy AP, Bowker MA, Ochoa-Hueso R. Species richness effects on ecosystem multifunctionality depend on evenness, composition and spatial pattern. *J Ecol.* 2012b; 100:317–330.
- Maestre FT, Cortina J. Insights into ecosystem composition and function in a sequence of degraded semiarid steppes. *Restor Ecol.* 2004; 12:494–502.
- Maestre FT, Cortina J. Ecosystem structure and soil-surface conditions drive the variability in the foliar $\delta^{13}C$ and $\delta^{15}N$ of *Stipa tenacissima* in semiarid Mediterranean steppes. *Ecol Res.* 2006; 21:44–53.
- Maestre FT, Escudero A. Is the patch size distribution of vegetation a suitable indicator of desertification processes? *Ecology.* 2009; 90:1729–1735. [PubMed: 19694122]
- Maestre FT, Puche MD, Guerrero C, Escudero A. Shrub encroachment does not reduce the activity of some soil enzymes in Mediterranean semiarid grasslands. *Soil Biol Biochem.* 2011; 43:1746–1749.
- Maestre FT, Quero JL, Gotelli NJ, Escudero A, Ochoa V, Delgado-Baquerizo M, García-Gómez M, Bowker MA, Soliveres S, Escolar C, García-Palacios P, et al. Plant species richness and ecosystem multifunctionality in global drylands. *Science.* 2012a; 335:214–218. [PubMed: 22246775]
- Maestre FT, Ramírez DA, Cortina J. Ecología del esparto (*Stipa tenacissima* L.) y los espartales en la Península Ibérica. *Ecosistemas.* 2007; 16:116–135.
- Maestre FT. On the importance of patch attributes, environmental factors and past human impacts as determinants of perennial plant species richness and diversity in Mediterranean semiarid steppes. *Divers Distrib.* 2004; 10:21–29.
- McClaran MP, Moore-Kucera J, Martens DA, van Haren J, Marsh SE. Soil carbon and nitrogen in relation to shrub size and death in a semi-arid grassland. *Geoderma.* 2008; 145:60–68.
- Millennium Ecosystem Assessment. *Ecosystems and Human Well-being: Synthesis.* Island Press; Washington, DC: 2005.
- Miranda JD, Padilla FM, Pugnaire FI. Response of a Mediterranean semiarid community to changing patterns of water supply. *Perspect Plant Ecol Evol System.* 2009; 11:255–266.
- Mouillot D, Villéger S, Scherer-Lorenzen M, Mason NWH. Functional structure of biological communities predicts ecosystem multifunctionality. *Plos One.* 2011; 6:e17476. [PubMed: 21423747]
- Naito AT, Cairns DM. Relationships between arctic shrub dynamics and topographically-derived hydrologic characteristics. *Environ Res Lett.* 2011; 6:045506.
- Noble, JC. *The Delicate and Noxious Scrub: CSIRO Studies on Native Tree and Shrub Proliferation in the Semi-arid Woodlands of Eastern Australia.* CSIRO; Lyneham, ACT: 1997.
- Paruelo JM, Garbulsky MF, Guerschman JP, Jobbagy EG. Two decades of normalized difference vegetation index changes in South America: Identifying the imprint of global change. *Int J Remote Sens.* 2004; 25:2793–2806.
- Pausas JG. Changes in fire and climate in the eastern Iberian Peninsula (Mediterranean basin). *Clim Change.* 2004; 63:337–350.

- Pearse IS, Hipp AL. Phylogenetic and trait similarity to a native species predict herbivory on non-native oaks. *Proc Natl Acad Sci USA*. 2009; 106:18097–18102. [PubMed: 19841257]
- Quinn, GP.; Keough, MJ. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press; 2002.
- Rangel TF, Diniz-Filho JAF, Bini LM. SAM: a comprehensive application for spatial analysis in macroecology. *Ecography*. 2010; 33:46–50.
- Ratajczak Z, Nippert JB, Collins SL. Woody encroachment decreases diversity across North American grasslands and savannas. *Ecology*. 2012; 93:697–703. [PubMed: 22690619]
- Reiss J, Bridle JR, Montoya JM, Woodward G. Emerging horizons in biodiversity and ecosystem functioning research. *Trends Ecol Evol*. 2009; 24:505–514. [PubMed: 19595476]
- Rogala JK, Hebblewhite M, Whittington J, White CA, Coleshill J, Musiani M. Human activity differentially redistributes large mammals in the Canadian Rockies National Parks. *Ecol Soc*. 2011; 16:16–36.
- Seifan M, Kadmon R. Indirect effects of cattle grazing on shrub spatial pattern in a mediterranean scrub community. *Basic Appl Ecol*. 2006; 7:496–506.
- Selene B, Collin SL. Shrub invasion decreases diversity and alters community stability in Northern Chihuahuan desert plant communities. *PLoS ONE*. 2008; 3:e2332. [PubMed: 18523686]
- Servicio del Esparto. *El esparto y su economía*. Ministerio de Industria, Comercio y Agricultura; Madrid: 1950.
- Soil Survey Staff. *Keys to Soil Taxonomy*. 6th edn. Pocahontas Press; Blacksburg: 1994.
- Soliveres S, Eldridge D, Maestre FT, Bowker MA, Tighe M, Escudero A. Microhabitat amelioration and reduced competition among understorey plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Persp Plant Ecol Evol System*. 2011; 13:247–258.
- Throop HL, Archer SR. Shrub (*Prosopis velutina*) encroachment in a semidesert grassland: spatial-temporal changes in soil organic carbon and nitrogen pools. *Global Change Biol*. 2008; 14:2420–2431.
- Tongway DJ, Sparrow AD, Friedel MH. Degradation and recovery processes in arid grazing lands of central Australia. Part 1: soil and land resources. *J Arid Environ*. 2003; 55:301–326.
- United Nations Environment Programme. *World Atlas of Desertification*. London, UK: Edward Arnold; 1992.
- Waide RB, Willig MR, Steiner CF, Mittelbach G, Gough L, Dodson SI, Juday GP, Parmenter RR. The relationship between productivity and species richness. *Annu Rev Ecol Syst*. 1999; 30:257–300.
- van Auken OW. Causes and consequences of woody plant encroachment into western North American grasslands. *J Environ Manage*. 2009; 90:2931–2942. [PubMed: 19501450]
- Verdú M, García-Fayos P. Nucleation processes in a Mediterranean bird-dispersed plant. *Funct Ecol*. 1996; 10:275–280.
- Wang G, Li H, An M, Jian N, Shengjun J, Wang J. A regional-scale consideration of the effects of species richness on above-ground biomass in temperate natural grasslands of China. *J Veg Sci*. 2011; 22:414–424.
- Wheeler CW, Archer SR, Asner GP. Climatic/edaphic controls on soil carbon/nitrogen response to shrub encroachment in desert grassland. *Ecol Appl*. 2007; 17:1911–1928. [PubMed: 17974331]
- White, RP.; Nackoney, J. *Drylands, people, and ecosystem goods and services: a web-based geospatial analysis*. World Resources Institute; Washington: 2003. Available at <http://pubs.wri.org>
- Whitford, WG. *Ecology of Desert Systems*. Academic Press; San Diego, CA: 2002.
- Villar R, Merino J. Comparison of leaf construction costs in woody species with differing leaf life-spans in contrasting ecosystems. *New Phytol*. 2001; 151:213–226.
- Zavaleta ES, Pasari JR, Hulvey KB, Tilman GD. Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. *Proc Natl Acad Sci USA*. 2010; 107:1443. [PubMed: 20080690]
- Zhang D, Hui D, Luo Y, Zhou G. Rates of litter decomposition in terrestrial ecosystems: global patterns and controlling factors. *J Plant Ecol*. 2008; 1:85–93.

Figure 1.

Effects of the presence of sprouting shrubs on multifunctionality (M), and on carbon (C), nitrogen (N) and phosphorous (P) cycling indices in grasslands (GR) and shrublands (SH). Data represent means $\pm$ SE ($n = 11$). ANOVA results for the data presented in this figure are shown in Supplementary Table S4.

Figure 2.

Effects of the presence of sprouting shrubs on the ten ecosystem functions studied in grasslands (GR) and shrublands (SH). Data represent means $\pm$ SE ($n = 11$). ANOVA results for the data presented in this figure are shown in Supplementary Table S5.

Figure 3.

Relative importance of predictor variables in models of ecosystem multifunctionality (M) and carbon (C), nitrogen (N), and phosphorus (P) indices. The height of each bar is the sum of the Akaike weights (W_i) of all models that included the predictor of interest, taking into account the number of models in which each predictor appears. Variable abbreviations as in Table 1.

Figure 4.

Relative importance of predictor variables in models of ecosystem functions of carbon , nitrogen and phosphorus: ORC = organic carbon, BGL = β -glucosidase extracellular activity, HEX = hexoses, PEN = pentoses, TON = total nitrogen, ATN = total available N, AMI = amino acids, PRO = proteins, AVP = available inorganic phosphorous, FOS = phosphatase extracellular activity. The height of each bar is the sum of the Akaike weights

(W_i) of all models that included the predictor of interest, taking into account the number of models in which each predictor appears. Variable abbreviations as in Table 1.

Table 1

Top five best-fitting regression models of ecosystem multifunctionality using ordinary least squares regression. Each column represents a different predictor variables (SR, perennial plant species richness; Enc I, encroachment index; SLO, slope; Clim-Ax1, first component of a principal-components analysis conducted with 21 climatic variables; Anth-Ax3, third component of a principal-components analysis conducted with the distance to the nearest town, the 1857, 1887 and 1950 population censuses of these towns and the distance to the closest dirty and asphalted road). AICc measures the relative goodness of fit of a given model; the lower its value, the more likely it is that this model is correct; AICc is the difference between the AICc of each model and that of the best model; Wi are the Akaike weights. Unshaded cells indicate variables that were not included in a particular model.

M								
SR	Enc I	SLO	Clim-Ax1	Anth-Ax3	R ²	AICc	AIC	Wi
					0.42	63.43	0	0.24
					0.39	69.44	0.01	0.23
					0.37	70.95	1.52	0.11
					0.43	71.87	2.44	0.07
					0.39	71.91	2.48	0.07
C								
SR	Enc I	SLO	Clim-Ax1	Anth-Ax3	R ²	AICc	AIC	Wi
					0.31	75.69	0	0.22
					0.34	76.53	0.85	0.14
					0.29	76.81	1.12	0.13
					0.32	77.74	2.05	0.08
					0.31	77.9	2.22	0.07
N								
SR	Enc I	SLO	Clim-Ax1	Anth-Ax3	R ²	AICc	AIC	Wi
					0.40	81.29	0	0.23
					0.35	81.91	0.62	0.17
					0.34	82.62	1.33	0.12
					0.40	83.45	2.16	0.08
					0.40	83.58	2.29	0.07
P								
SR	Enc I	SLO	Clim-Ax1	Anth-Ax3	R ²	AICc	AIC	Wi
					0.34	99.93	0	0.26
					0.29	100.55	0.62	0.19
					0.34	102.38	2.45	0.08
					0.26	102.6	2.66	0.07
					0.34	102.6	2.67	0.07

Table 2

Multi-model averaged parameters estimates of the analyses conducted with the multifunctionality index (M) as dependent variable and all possible combination of the independent variables used (SR, perennial plant species richness; Enc I, encroachment index; SLO, slope; *Clim-Ax1*, first component of a principal-components analysis conducted with 21 climatic variables; *Ant-Ax3*, third component of a principal-components analysis conducted 6 variables related with anthropic factors). The results shown are the parameter estimates averaged across 31 models using Akaike weights and ordinary least square regression (A) and the parameters estimates for the best (that with the lowest AIC_c) and most parsimonious (fewest independent variables with $AIC < 2$) ordinary least squares models (B and C, respectively). STE = Standardized coefficient, SE = Standard error and CI = confidence intervals.

A						
Variable	Coefficient	STE	SE	t	95% Lower CI	95% Upper CI
SR	0.018	0.250	0.006	2.959	0.006	0.030
Enc I	0.678	0.507	0.175	3.867	0.334	1.021
SLO	0.001	0.015	0.003	0.510	-0.004	0.007
<i>Clim-Ax1</i>	0.011	0.043	0.009	1.175	-0.007	0.029
<i>Ant-Ax3</i>	0.183	0.296	0.064	2.867	0.058	0.309
B						
Variable	Coefficient	STE	SE	t	95% Lower CI	95% Upper CI
SR	0.015	0.205	0.009	1.549	-0.004	0.033
Enc I	0.671	0.503	0.164	4.094	0.35	0.993
<i>Ant-Ax3</i>	0.16	0.258	0.081	1.97	<0.001	0.318
C						
Variable	Coefficient	STE	SE	t	95% Lower CI	95% Upper CI
Enc I	0.723	0.542	0.163	4.432	0.404	1.042
<i>Ant-Ax3</i>	0.209	0.338	0.076	2.763	0.060	0.358