Plant interactions govern population dynamics in a semi-arid plant community

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Summary

1 The structure and composition of plant communities are influenced by positive and negative interactions between plants, the balance of which may change in intensity and sign through time and space, depending on availability of resources and on plant life history.

2 Over a 2-year period we analysed the balance of interactions between different life stages of a perennial grass, *Stipa tenacissima*, and a shrub, *Cistus clusii*, the dominant species in a semi-arid community in south-east Spain.

3 *Cistus* shrubs acted as nurses for juvenile *Stipa* plants, improving their water status, nutrient content, carbon assimilation rates and growth. The mechanisms underlying this facilitation effect were mainly the improvement of microclimatic conditions and soil physical and chemical properties under shrub canopies. By contrast, juvenile *Stipa* plants had an overall neutral effect on *Cistus* shrubs, although *Cistus* suffered some competitive effects during periods of water shortage. At this life stage, the short-term outcome of the interaction for both species was dependent on resource availability.

4 Close spatial association between adult plants had no negative effects for the inter-acting species, although positive effects most likely counterbalanced negative effects.

5 The long-term outcome of the interaction is reflected in the spatial distribution of both species, and determines population dynamics in this semi-arid plant community.

6 Our data show that the short-term balance of plant interactions may easily shift in response to environmental variability, which in turn may have important consequences for plant community structure.

Key-words: competition, environmental variability, facilitation, nurse syndrome

Introduction

Plant interactions are key processes that strongly influence the composition and structure of plant communities (Roughgarden & Diamond 1986). These interactions commonly encompass positive and negative effects operating simultaneously and bidirectionally (Aguiar *et al*. 1992; Pugnaire *et al.* 1996b; Holzapfel & Mahall 1999), the final outcome being the net balance between all of these effects (Callaway & Walker 1997; Holmgren

et al. 1997). This balance is dynamic and may change depending on life-history stage (Valiente-Banuet *et al*. 1991; Aguiar *et al*. 1992; Pugnaire *et al.* 1996c; Rousset & Lepart 2000) or the intensity of abiotic severity (Bertness & Callaway 1994; Holzapfel & Mahall 1999; Tielbörger & Kadmon 2000; Tewksbury & Lloyd 2001; Callaway *et al*. 2002).

An example of this shifting balance is often seen in the so-called 'nurse plant syndrome' (Franco & Nobel 1988), in which adult plants of one species facilitate the establishment of seedlings of another species. As seedlings become adults they may compete with or even out-compete the benefactor species (Valiente-Banuet *et al*. 1991; Flores-Martinez *et al*. 1994; Barnes & Archer 1999; Rousset & Lepart 2000; Anderson *et al*. 2001; Gasque & García-Fayos 2004), although in some cases the nurse

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plant continues to benefit adults of the subordinate species (Tirado & Pugnaire 2003) and mutualistic relationships may even develop between facilitator and facilitated species (Pugnaire *et al.* 1996b,c).

The nurse plant syndrome is now a well-documented phenomenon in a broad range of habitats, from arid and semi-arid shrublands and steppes (Tielbörger & Kadmon 2000; Tewksbury & Lloyd 2001) to savannas (Barnes & Archer 1999; Anderson *et al.* 2001), wetlands and marshes (Egerova *et al.* 2003), coastal dune systems (Shumway 2000; Tirado & Pugnaire 2003), Mediterranean forests and shrublands (Rousset & Lepart 2000; Verdú & García-Fayos 2003), and alpine systems (Nunez *et al.* 1999; Cavieres *et al.* 2002).

The nurse plant syndrome seems to be critical in dry habitats. More than half of these examples come from such environments (Flores & Jurado 2003), supporting the general hypothesis that facilitation increases in importance with increasing environmental severity (Bertness & Callaway 1994; Callaway & Walker 1997; Brooker & Callaghan 1998). In semi-arid environments, facilitation usually involves increased water or nutrient availability (Holzapfel & Mahall 1999). The soils under nurse plants commonly have a higher nutrient content than the surrounding open areas, and thus induce a relative increase in seedling growth (García-Moya & McKell 1970; Pugnaire *et al.* 1996a, 2004; Tirado & Pugnaire 2003). In addition, the shade from nurse plants reduces thermal amplitudes and decreases soil water evaporation (Domingo *et al.* 1999), which may further facilitate germination of seeds and growth of seedlings. Shade also reduces thermal stress and transpiration of understorey plants, protecting them from photo-inhibition (Nobel 1980; Fuentes *et al.* 1984; Vetaas 1992; Moro *et al.* 1997).

In these xeric systems, however, shrubs may also simultaneously impair performance of understorey plants through competition for water (see review in Fowler 1986) and reduced light availability (Holmgren *et al.* 1997; Forseth *et al.* 2001; Hastwell & Facelli 2003). Facilitation then occurs when the benefits of improved water relations or enhanced nutrient availability exceed the effect of competition for water and light limitation (Holmgren *et al.* 1997). Under wetter conditions, competition for light may be overriding (Holmgren *et al.* 1997; Forseth *et al.* 2001).

Water is one of the most limiting factors for plant growth in semi-arid environments and regulates many ecological processes, not only because of its scarcity but also because of its variability in space and time and the unpredictability of rainfall events (Ehleringer *et al.* 1999; Lázaro *et al.* 2001; Schwinning & Sala 2004; Sher *et al.* 2004). It can therefore be hypothesized that the direction and intensity of plant interactions will vary as a function of the temporal fluctuation (intra- and interannual) of water availability. However, few studies have experimentally analysed changes in the net balance of plant interactions induced by climatic conditions, and still fewer have focused on the impacts of the pulsed availability of

resources such as water (but see Holzapfel & Mahall 1999; Tielbörger & Kadmon 2000; Ludwig *et al.* 2003).

Moreover, some important aspects of the plant nurse syndrome affecting plant community dynamics are not completely documented. Most of the above-mentioned studies ran for only a limited time (e.g. Rousset & Lepart 2000) and few addressed the long-term effects of the nurse plant (Barnes & Archer 1999; Rousset & Lepart 2000; Gasque & García-Fayos 2004), by continuing to monitor success beyond the seedling stage (Barnes & Archer 1999) or by analysing the effects of the facilitated plant on the nurse.

In this study, we analysed temporal changes in the balance of the interactions between a perennial tussock grass species, *Stipa tenacissima* L., and a small shrub, *Cistus clusii* Dunal, in a semi-arid community of SE Spain, hypothesizing that:

1 the net interaction comprised positive and negative effects acting bidirectionally;

2 the net balance of these effects would change with changes in the life history of *Stipa*;

3 the net balance changes temporally as a function of water stress experienced by plants; and

4 the long-term effect of the interaction is mirrored by the spatial distribution of both species.

Plant responses to the removal of neighbours were tested over a period of 2 years and the response of target plants was related to their life-history stage, variation in key environmental parameters and the age of neighbours. Plant responses were analysed at different temporal scales: short-term responses to water availability were assessed by physiological measurements, mid-term responses through plant growth and reproductive output and long-term effects by analysing spatial distribution patterns.

Methods

STUDY SITE AND SPECIES

The field site is located in the northern foothills of the Sierra Alhamilla range, Almería Province, Spain (37°01′ N, 2°23′ W, 430 m elevation). The climate is semi-arid with a mean annual precipitation of 230 mm and a pronounced dry season from June to September, during which there is no rain in most years. Site and climate characteristics have been described elsewhere (Pugnaire & Haase 1996; Balaguer *et al.* 2002). The site is a steppe-like habitat with 35% plant cover (Sánchez 1995) dominated by the perennial grass *Stipa tenacissima* and some scattered shrubs, being *Cistus clusii* the most abundant woody species.

Stipa tenacissima (Poaceae) is a long-lived perennial grass that forms large tussocks (~0.5 m^2) and spreads both by seeds and vegetatively. It has long, narrow leaves, up to 100 cm long and 2–3 mm wide, which can fold along their long axes and curl up during periods of water deficit. Root : shoot ratio is low (Sánchez 1995) and roots spread both superficially and up to 1 m deep.

Cistus clusii (Cistaceae) is a multibranched sclero-phyllous shrub, growing up to 1 m tall, typical of the Mediterranean region. It has a life span of about 15–20 years (Munné-Bosch & Alegre 2002) and reproduces only by seed (Pugnaire & Lozano 1997). It has linear hypostomatic leaves (dark green above, white-tomentose beneath), which can curl up, thereby reducing interception of solar radiation (Munné-Bosch *et al.* 2003). Fruits are capsules 4–8 mm wide. Like other *Cistus* species it is an early colonizer and, as the most drought-resistant species of *Cistus* (Polunin & Smithies 1973), it is adapted to the spectrum of unpredictable disturbances that characterize Mediterranean ecosystems.

PLANT SPATIAL DISTRIBUTION ANALYSIS

We analysed the spatial distribution pattern of *Cistus* and *Stipa* plants using Ripley's *K* function (Ripley 1976; Haase 1995) in a 20×20 m field plot. Ripley's *K* function is generally considered the best technique for bivariate point pattern analysis (Dale 1999). It considers each plant as the central point of a circle of radius *t* and counts the number of individuals found within the circle. We used the weighting approach to correct for edge effects (Haase 1995). If the distribution of the points is Poisson random, the expected value of the cumulative function $K(t)$ equals πt^2, i.e. the area of the circle. For easier interpretation, the derived sample statistic $(\sqrt{K[(t)/\pi]} - t)$ is generally plotted, as this expression has a zero expectation for any value of *t* when the pattern is Poisson random, being positive when it is aggregated and negative when regular (uniform).

Plants of both species were classified into two categories as a function of life stage and plant size. We considered as juvenile those *Stipa* plants that had not developed spikes and had a basal diameter of < 10 cm. All measured *Cistus* plants had old inflorescences, but analysis of the population height frequency histogram suggested division into small (< 62 cm tall) and large plants.

EXPERIMENTAL DESIGN

In January 2000 we randomly selected 30 juvenile *Stipa* plants located beneath small *Cistus* (*Stipa–Cistus* 'pairs') and 10 plants of each species living in isolation. We removed *Stipa* plants from 10 pairs and *Cistus* from another 10 pairs, leaving the remaining pairs intact. *Cistus* sizes were determined by measuring plant height and maximum and minimum diameter. For *Stipa* we measured basal diameter, height and number of tillers. At the end of the experiment (August 2002) we removed all plants from the field, placed them in an oven at 70 °C for 48 h in the laboratory and measured their dry mass.

In March 2000 we applied the same experimental treatments to pairs of adult *Stipa* and large *Cistus* plants and also selected adult *Stipa* and large *Cistus* plants living in isolation. For each species we selected plants of similar size and measured their height, maximum diameter and its perpendicular.

MICROMETEOROLOGICAL MEASUREMENTS AND SOIL PROPERTIES

Air temperature and relative humidity 10 cm above the soil were recorded every 5 min for 1 week during July 2002 with microclimatic sensors (HOBO H8, Onset Computers, Pocasset, MA, USA). Sensors were placed above bare soil and beneath *Stipa* tussocks and *Cistus* canopies of both isolated plants and those living in pairs. Photosynthetically active radiation (PAR, 400–700 nm) levels were recorded every 5 min over the course of one sunny day in September 2002. PAR levels were recorded using a quantum sensor (SKP215, Skye Instruments Ltd, Llandrindod Wells, UK) placed beneath the geometrical centre of *Cistus* shrubs, and also above bare soil, and connected to HOBO H8 data loggers.

Nine ~200-cm^3 soil samples were collected in August 2002 from the upper 10 cm of soil under juvenile tussocks and small shrubs. Each sample was thoroughly homogenized and air-dried in the laboratory. A known volume of soil was weighed to determine bulk density. Soils were sieved through a 2-mm mesh, and analysed to determine soil organic matter by wet digestion (Porta *et al.* 1986), total soil nitrogen (N) by Kjeldahl analysis with $SeSO_4$–K_2SO_4 catalyst in a semi-automatic analyser (Velp Científica s.r.l., Italy), and soluble phosphorous (P) extracted by the Watanabe & Olsen (1965) method and determined by colorimetry with a spectrophotometer (Bausch & Lomb, Rochester, NY, USA).

ALLOMETRIC AND PHYSIOLOGICAL MEASUREMENTS

In June 2000 we sampled the first mature, fully developed leaf from three tillers of nine *Stipa* tussocks and from nine different 1-year-old twigs of every *Cistus* shrub. We resampled in an identical fashion in June 2001 and 2002. Each leaf was dried at 70 °C for 72 h. For *Stipa* leaves, length and width (1 cm above the leaf base) were recorded, and leaf dry mass and leaf area were recorded for both species. We measured the area of *Cistus* leaves from scanned leaf images using an image area analyser (Midebmp, Ramón Ordiales, Almería). For *Stipa* leaves we selected a 10-cm segment in the central part of the leaf and measured its width. The dry mass of samples was then determined and the specific leaf area (SLA) calculated.

In June 2001 and 2002 we measured fruit production on three stems of each *Cistus* shrub and counted the number of flower spikes on each mature *Stipa* tussock. We selected three mature capsules (fruits) from each *Cistus* shrub and 50 seeds from each *Stipa* tussock, oven-dried them at 70 °C for 72 h and weighed them. In June 2002 we also selected three spikes per *Stipa* plant and recorded their length and total seed dry mass.

In July 2002 we measured pre-dawn water potential (Ψ_{pd}) on leaves (*Stipa*) and terminal shoots (*Cistus*) using a pressure chamber (SKPM 1400, Skye Instruments Ltd). We used twigs of *Cistus* because, in woody

species, these are more representative of water status than are leaves (McCutchan & Shackel 1992). Pre-dawn water potential was also measured in August 2002. The photochemical efficiency of photosystem II (*Fv/Fm*) was measured at dawn with a portable fluorimeter (PEA, Hansatech, Kings Lynn, UK) that used pulses of ~3000 μmol m^{-2} s^{-1}. Prior to measurement leaves were dark-adapted for 30 min. Leaf conductance to water vapour (*gs*) and photosynthetic rate (*A*) were measured on green, mature leaves that faced east and were fully illuminated by the sun during the early morning hours (07:00–08:30 solar time, i.e. at the time of maximum photosynthetic rates; Haase *et al.* 1999; Munné-Bosch *et al.* 2003). Measurements were made with a portable infrared gas analyser (LCi, Analytical Development Company Ltd, Hoddesdon, UK) under ambient CO_2 concentrations. Measurements were expressed on a projected leaf area basis. In *Stipa* we used Cregg's (1992) correction for cylindrical leaves. In July and August 2002 leaf samples were collected and dried in an oven at 70 °C for 72 h, ground with a mill (Fritsch Pulveriseke, Germany) and analysed for total N content.

STATISTICAL ANALYSES

Differences between treatments and life stages were tested for each species separately using ANOVA at a significance level of 0.05. One-way ANOVA was used for individual measurements, and RM-ANOVA or M-ANOVA (if the former was not applicable) for time-repeated measurement analysis. Homogeneity of variances was checked using Levene's test, and the Box M test was used to check the homogeneity of variance/covariance matrices and the sphericity and compound symmetry in RM-ANOVA. *Post-hoc* differences were explored with Scheffé's test. When variables were heterocedastic we applied the alternative non-parametric test for one-way ANOVA.

Seed and fruit biomass were log-transformed to normalize their distribution.

Data were analysed with STATISTICA 6.0 software (StatSoft Inc., Tulsa, OK, USA). Data are presented as means ± 1 SE throughout.

For spatial data we used the SPPA program (Haase 2001).

Results

SPATIAL DISTRIBUTION

Cistus and *Stipa* differed in their spatial distribution depending on plant size and life-history stage. At both stages, the two species were spatially associated at small distances. The aggregated pattern at close distances was due to the close spatial association of juveniles of *Stipa* to shrubs (aggregated at distances < 2.5 m, regular above 6 m; Fig. 1a, *P* < 0.05), because mature *Stipa* and large *Cistus* were only aggregated for distances closer than 0.6 m (Fig. 1b, *P* < 0.05) and were distributed randomly for greater distances.
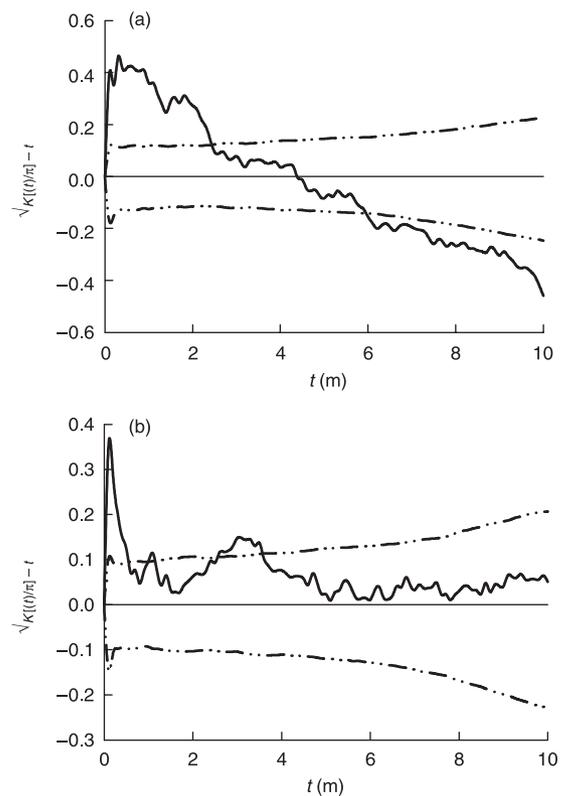

Fig. 1 Second-order spatial analysis of the distribution pattern of (a) small *Cistus clusii* and juvenile *Stipa tenacissima* plants and (b) large *Cistus clusii* plants and mature *Stipa tenacissima* tussocks. Each plot represents the derived statistic of Ripley's *K* function vs. neighbours distance *t*. Positive values indicate aggregation, and negative values regularity. Dotted lines give 95% confidence intervals for complete spatial randomness.

SOIL PROPERTIES AND MICROCLIMATIC CONDITIONS

In general soils were relatively infertile, but organic matter and nutrient levels were higher under plant canopies (Fig. 2). Soils under pairs from which *Stipa* had been removed (i.e. C–S) had the highest nutrient content whereas soil from under isolated *Stipa* had the lowest values, which were always similar to those in open areas (Fig. 2). Compared with soils from bare areas, soils where both species grew together had higher organic matter, and N and P content (Fig. 2).

Microclimatic conditions were less severe, and incident PAR lower, under small *Cistus* shrubs than in gaps or under juvenile *Stipa* living alone (Fig. 3). There were no differences in the microclimatic conditions beneath *Cistus* shrubs living alone and those growing with *Stipa* (Fig. 3a,b). Daily temperature amplitude was clearly larger in gaps and under isolated juvenile *Stipa* than under shrubs, and mean and maximum temperature were 5 °C and 10 °C higher in gaps than under shrub canopies (Fig. 3a). Relative humidity was higher in gaps at night and similar to other situations during the day (Fig. 3b). Although there was a reduction of incident PAR by ~15% beneath shrub canopies compared with gaps, PAR levels were still non-limiting (Fig. 3c).
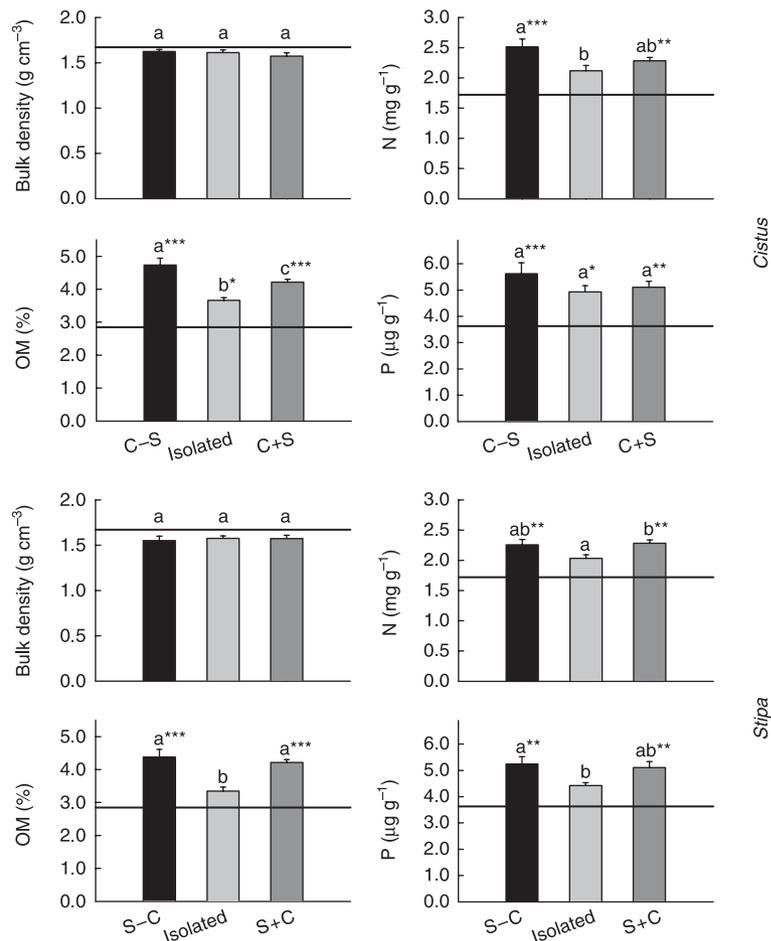
Fig. 2 Soil properties beneath small *Cistus* and juvenile *Stipa* (bars) and bare ground (line). Data are mean values ± 1 SE, *n* = 8. Bars with different letters are significantly different at *P* < 0.05. Asterisks indicate significant differences between treatment and bare soil (*, **, ***, at *P* < 0.05, 0.01 and 0.001, respectively). Treatments legend: (C−S) *Cistus* with *Stipa* removed; (Isolated) *Cistus* isolated; (C+S) *Cistus* associated with *Stipa*. For *Stipa*: (S−C) *Stipa* with *Cistus* removed; (Isolated) *Stipa* isolated; (S+C) *Stipa* associated with *Cistus*.

Table 1 Size (height and number of tillers per plant) of juvenile *Stipa* plants under each treatment at the beginning (2000) and end (2002) of the experiment. Data are mean values ± 1 SE, *n* = 8. Different letters for a variable indicate significant differences between treatments. *F* values from ANOVA and their significance are shown in the last column (*, **, ***, at *P* < 0.05, 0.01, 0.001, respectively; ns = not significant)

	Stipa isolated	*Stipa* (−*Cistus*)	*Stipa* (+*Cistus*)	$F_{2,21}$
2000				
Height (cm)	35.1 ± 3.6^a	65.8 ± 2.5^b	62.5 ± 3.8^b	25.0***
Tiller no.	30.2 ± 1.8^a	18.4 ± 2.9^b	17.4 ± 2.7^b	7.9**
2002				
Height (cm)	37.4 ± 1.8^a	45.7 ± 3.5^a	57.3 ± 3.0^b	12.3***
Tiller no.	47.0 ± 4.0^a	48.0 ± 7.9^a	44.4 ± 6.3^a	0.1^{ns}
Biomass (g)	31.0 ± 4.3^a	45.2 ± 8.7^{ab}	63.2 ± 9.6^b	4.2*

PLANT SIZE, LEAF SIZE AND REPRODUCTIVE OUTPUT

Juvenile *Stipa* plants associated with *Cistus* were taller than isolated plants (Table 1). After 31 months, juvenile plants living under the protection of *Cistus* were taller and their biomass twice that of isolated plants (Table 1), whereas *Stipa* plants from pairs with *Cistus*

removed had intermediate height and biomass values. Mature *Stipa* plants had consistent sizes throughout the experiment ($F_{4,40} = 0.2$, $P = 0.9$), with a mean height of 84.2 ± 1.4 cm and a projected area of 0.48 ± 0.04 m².

Mature leaves of adult *Stipa* tussocks were bigger and had a lower SLA than those of juvenile tussocks (Fig. 4). At the beginning of the experiment, leaves of juvenile *Stipa* growing alone were smaller and thinner
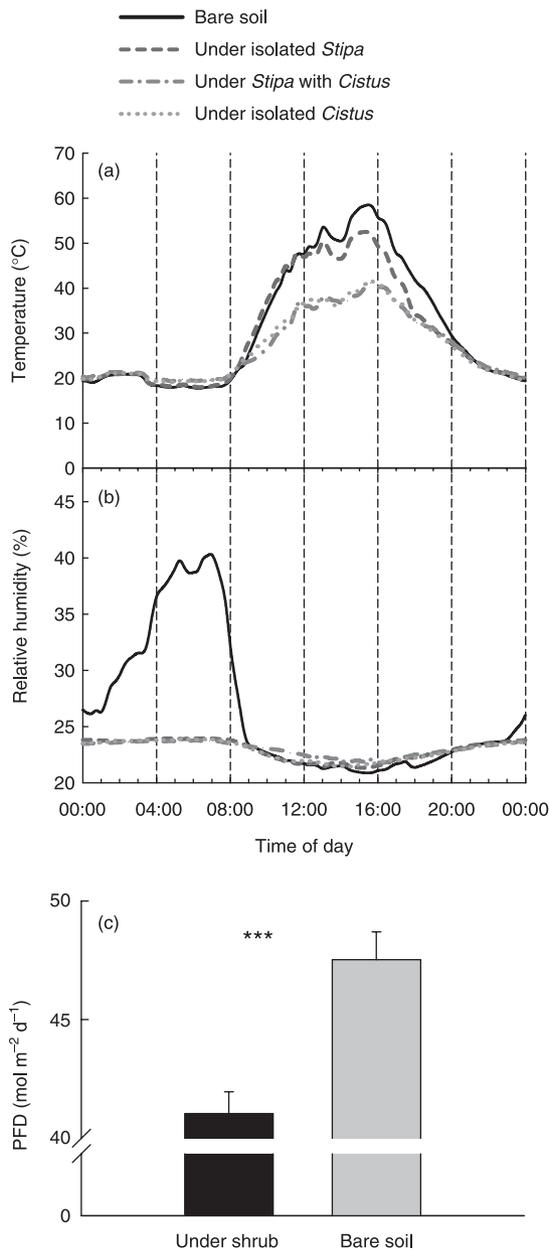
Fig. 3 Air temperature (a) and relative humidity (b) in bare soil and beneath juvenile *Cistus* and *Stipa* canopies between June and July 2002. Photosynthetic photon flux density (PFD) (c) beneath juvenile *Cistus* and in bare soil in September 2002. Data are mean values ± 1 SE (*n* = 2 on three sunny days). Asterisks indicate significant differences at *P* < 0.001.

Large *Cistus* shrubs had double the projected area of small shrubs (0.34 ± 0.04 m^2 vs. 0.17 ± 0.02 m^2, $F_{1,42}$ = 10.5, *P* < 0.003), had greater canopy volume (1.9 ± 0.03 m^3 vs. 0.7 ± 0.08 m^3, $F_{1,42}$ = 16.4, *P* < 0.001) and were taller (75.8 ± 3.2 cm vs. 56.4 ± 2.0 cm, $F_{1,42}$ = 27.4, *P* < 0.001). However, there were no further differences in size, biomass or leaf properties between shrub sizes or treatments (data not shown). The only notable difference was that, as for mature *Stipa* plants, *Cistus* leaves were in general heavier in 2002 (8.0 ± 0.2 mg) than in 2000 (3.9 ± 0.1 mg) and 2001 (5.0 ± 0.2 mg, $F_{2,84}$ = 191.0, *P* < 0.001).

There were no differences between treatments or years in spike production of mature *Stipa* tussocks (7.9 ± 1.1 in 2001 and 10.8 ± 1.7 in 2002, $F_{2,18}$ = 0.4, *P* = 0.70) or seed mass ($F_{2,12}$ = 0.5, *P* = 0.62), which averaged 5.2 ± 0.17 mg.

In 2001, fruit production of large *Cistus* shrubs was higher than small shrubs (54 ± 5.8 vs. 35.4 ± 4.4 capsules per stem, respectively, $F_{1,30}$ = 6.9, *P* < 0.02) but there were no differences in 2002, when fruit production was double that of 2001 (99 ± 8.3 vs. 44.6 ± 3.9 capsules per stem, $F_{1,30}$ = 32.7, *P* < 0.001). In general, capsule mass was also heavier in large shrubs than in small shrubs (29.9 ± 0.7 mg vs. 27.1 ± 0.7 mg, $F_{1,42}$ = 13.3, *P* < 0.001) and capsules were, on average, heavier in 2002 than in 2001 (31.2 ± 0.6 mg vs. 25.7 ± 0.6 mg, $F_{1,42}$ = 58.7, *P* < 0.001). There were no other notable differences in *Cistus* reproductive output.

PHYSIOLOGICAL RESPONSES

Unseasonal rains in July 2002 reduced plant water deficit in early summer, and both species displayed high physiological rates (Figs 5–7), with values close to their field optimum in periods of non-water deficit (Balaguer *et al.* 2002; Munné-Bosch & Alegre 2002).

Small *Cistus* shrubs had higher *gs* and *A* than large ones (Table 2, Fig. 5a) and higher *Fv/Fm* (0.820 ± 0.003 vs. 0.804 ± 0.006, Table 2). Leaf N content was similar in both groups (*P* = 0.11, Fig. 6a). There were no other notable differences in these physiological variables (Table 2).

Juvenile *Stipa* tussocks displayed higher *gs* and *A* than mature tussocks (Table 2, Fig. 5b). In each size class all treatments displayed similar *gs* values (Table 2, Fig. 5b), and for mature tussocks there was no difference in *A* between treatments (Fig. 5b). Isolated juvenile *Stipa* tussocks had lower *A* values than plants in the other treatments (Fig. 5b).

Juvenile and mature *Stipa* plants displayed similar *Fv/Fm* values (0.774 ± 0.003, Table 2). Independent of life-history stage, *Stipa* plants associated with *Cistus* displayed higher *Fv/Fm* values than *Stipa* with *Cistus* removed (0.787 ± 0.005 vs. 0.762 ± 0.005, *P* < 0.01), whereas isolated *Stipa* plants displayed intermediate values (0.775 ± 0.005).

Leaf N content was higher in mature than in juvenile *Stipa* plants (7.1 ± 0.8 mg g^{-1} vs. 6.7 ± 0.8 mg g^{-1}, Table 2). Juvenile *Stipa* plants associated with *Cistus* had twice

than those of *Stipa* associated with *Cistus* (Fig. 4). These differences persisted throughout the experiment. However, by the end of the experiment the biomass and length of leaves of *Stipa* from pairs with *Cistus* removed became significantly smaller than leaves of juvenile *Stipa* associated with *Cistus* (Fig. 4).

Leaves from mature *Stipa* tussocks had similar size in all treatments (Fig. 4). Leaf mass and length were higher in general in 2002 than in other years ($F_{2,42}$ = 9.0 and 247.5 for leaf mass and length, respectively, *P* < 0.001 in both cases, Fig. 4), whereas SLA values remained constant over time (Fig. 4).
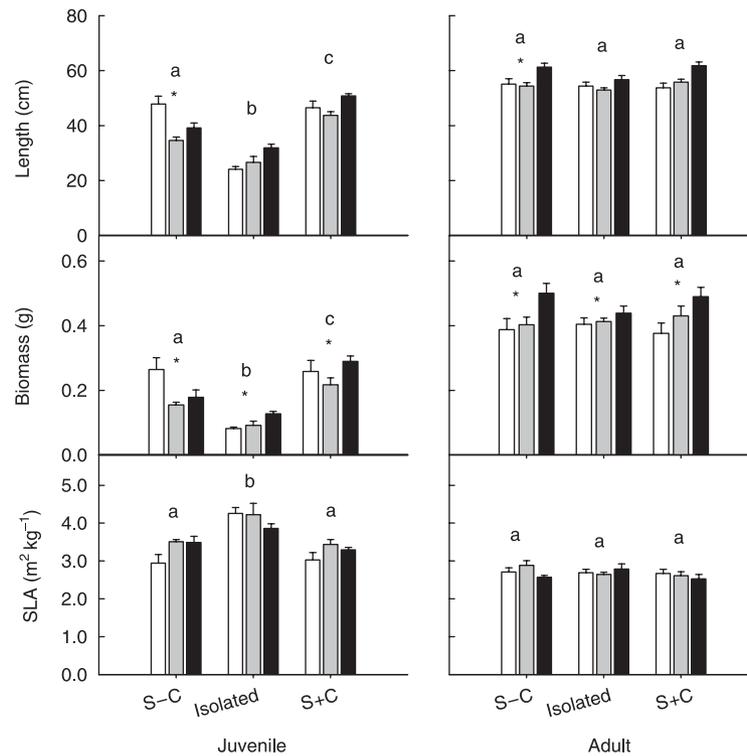
Fig. 4 Leaf length, mass and specific leaf area (SLA) of *Stipa* leaves in 2000 (clear bars), 2001 (grey) and 2002 (solid). Data are mean values ± 1 SE, *n* = 8. Bars with different letters are significantly different at *P* < 0.01. Asterisks indicate significant differences between years at *P* < 0.05. Treatments as in Fig. 2.

Table 2 *F* values from MANOVA and significance (*, **, ***, at *P* < 0.05, 0.01, 0.001, respectively; ns = not significant) of differences between different sizes and treatments of *Cistus* and *Stipa* for the following variables: conductance to water vapour (*gs*), photosynthetic rate (*A*), dawn photochemical efficiency of PSII (*Fv/Fm*), July pre-dawn water potential (Ψ_{pd} – Jul) and nitrogen content (N) of leaves and pre-dawn water potential (Ψ_{pd} – Ag) in August 2002. The number of replicates was seven except for leaf nitrogen content (8) and *Fv/Fm* (6)

	Cistus			*Stipa*		
	Size $F_{1,36}$	Treatment $F_{2,36}$	S × T $F_{2,36}$	Size $F_{1,36}$	Treatment $F_{2,36}$	S × T $F_{2,36}$
gs	51.0***	2.3ns	1.4ns	30.3***	1.5ns	3.4*
A	70.5***	0.5ns	1.7ns	22.4***	5.5**	6.7**
Fv/Fm	5.4*	2.3ns	1.4ns	0.2ns	6.1**	1.3ns
Ψ_{pd} – Jul	6.5*	12.2***	19.9***	36.7***	19.8***	16.2***
N	1.7ns	2.3ns	1.2ns	4.2*	5.5**	4.7*
Ψ_{pd} – Ag	21.3***	9.0***	5.8**	2.5ns	0.2ns	0.5ns

the N content of the other treatments, whereas isolated plants had the lowest (Fig. 6b).

WATER STATUS

In July 2002, when water availability was high due to unseasonal rains, small plants had lower Ψ_{pd} than large plants (Fig. 7). In both species large plants responded similarly to treatments (Fig. 7), and small, isolated plants showed the lowest water potential values (Fig. 7). Juvenile *Stipa* associated with *Cistus* had the highest water potentials (Fig. 7b).

As the summer advanced both species experienced progressive reduction in water availability. Ψ_{pd} dropped drastically in August and all plants displayed an aver-

age value of −5.37 ± 0.05 MPa, this being two times lower than those in July (−2.30 ± 0.06 MPa, *P* < 0.01). All *Stipa* plants had similar Ψ_{pd} in August (Fig. 7b) as did *Cistus* plants, except for small shrubs associated with *Stipa* whose Ψ_{pd} were lowest (Fig. 7a).

These results suggest that the water status of large *Cistus* shrubs and adult *Stipa* tussocks was unrelated to neighbour presence, irrespective of the seasonal water availability in the soil. By contrast, the water status of small plants was improved by association with other species, but only when the overall water deficit was not high. For small *Cistus* shrubs this improvement seems to be caused by changes in soil properties alone (Fig. 7a) whereas facilitation of juvenile *Stipa* seems to be due to both microhabitat amelioration and improvement of
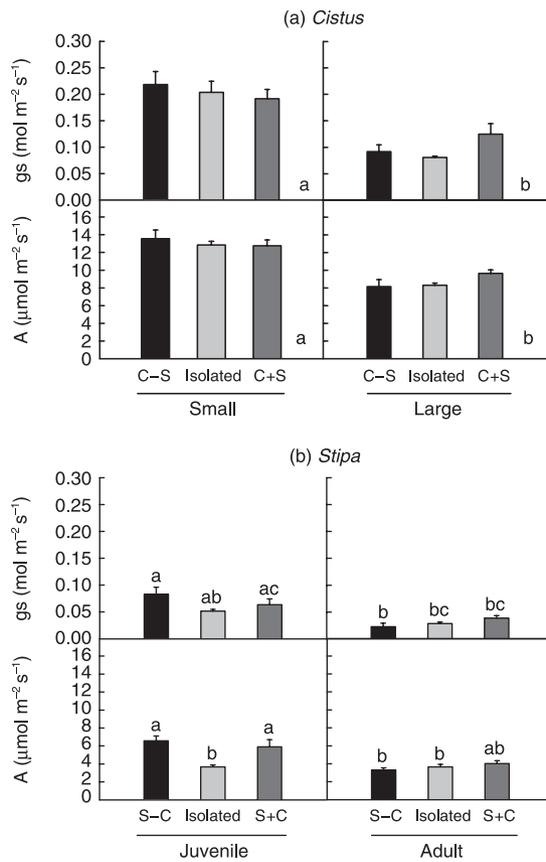
Fig. 5 Leaf conductance to water vapour (*gs*) and photosynthetic rate (*A*) of *Cistus* (a) and *Stipa* (b). Data are mean ± 1 SE, *n* = 7. For each species, bars with different letters are significantly different at *P* < 0.05. Treatments as in Fig. 2.
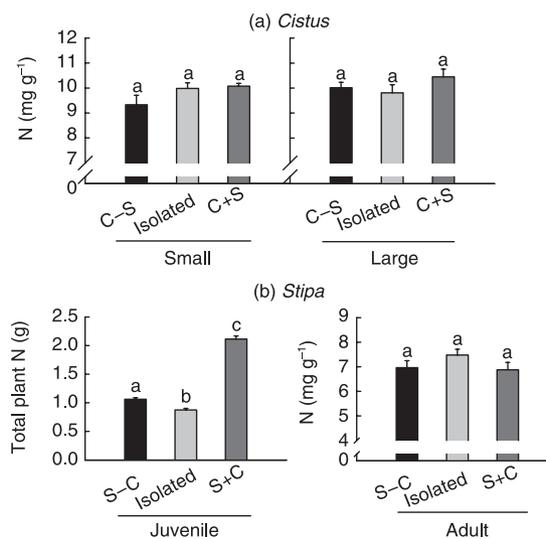

Fig. 6 Leaf nitrogen content of small and large *Cistus* shrubs (a) and juvenile and mature *Stipa* tussocks (b). For juvenile *Stipa*, data represent nitrogen content per plant. Data are mean ± 1 SE, *n* = 8. For each species, bars with different letters are significantly different at *P* < 0.01. Treatments as in Fig. 2.

Fig. 7 Pre-dawn water potentials (Ψ_{pd}) of *Cistus* twigs (a) and *Stipa* leaves (b) in July (solid) and August (grey bars) in 2002. Data are mean ± 1 SE, *n* = 7. For each species, bars with different letters are significantly different at *P* < 0.01. Treatments as in Fig. 2.

Discussion

Our results highlight the existence of both positive and negative interactions between *Cistus clusii* and *Stipa tenacissima*, the balance of which changes with plant life-history stage and which, in the long term, strongly influence population structure in this semi-arid community. *Cistus* facilitated juvenile *Stipa*, whereas the overall effect of juvenile *Stipa* on *Cistus* was negligible. However, these results suggest that the short-term balance of this interaction is variable and depends on environmental conditions, particularly water availability. By contrast, when growing in close association, mature *Cistus* and *Stipa* plants did not have any noticeable effect on one another.

INTERACTION BALANCE BETWEEN SHRUBS AND UNDERSTOREY *STIPA*

Around 62% of juvenile *Stipa* plants were spatially associated with *Cistus* (χ^2 = 4.8, *P* < 0.05, Fig. 1a),

soil properties (Fig. 7b). When water deficit was high, however, positive effects of *Cistus* on *Stipa* disappeared (Fig. 7b) and small *Cistus* shrubs suffered from competition with *Stipa* for water (Fig. 7a).
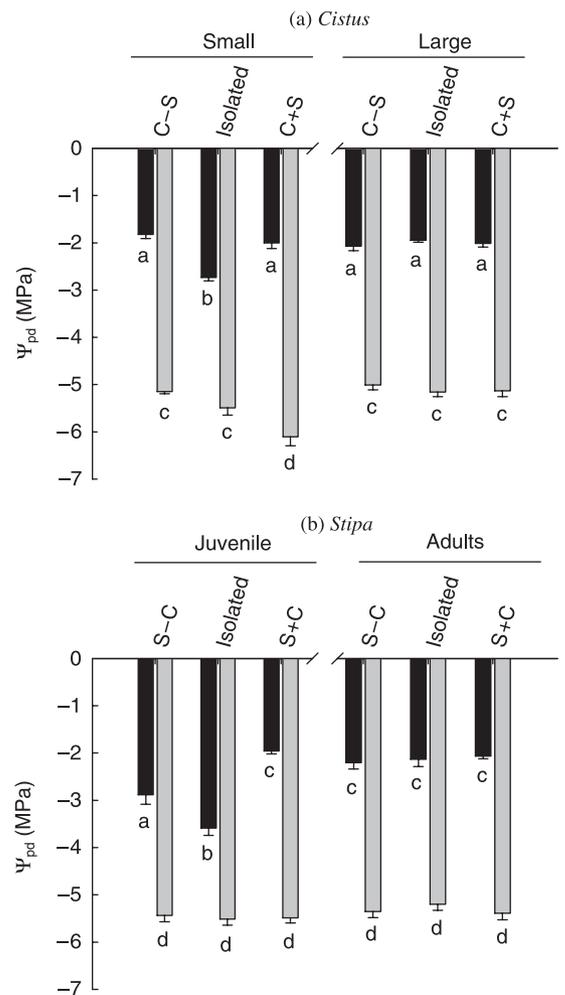
suggesting that shrubs facilitated *Stipa* seedlings. Our physiological and functional measurements support this idea, as juvenile *Stipa* plants associated with *Cistus* had enhanced performance in numerous ways, including increased plant and leaf biomass (Fig. 4), gas exchange rates (Fig. 5b), nitrogen pool (Fig. 6b) and, under slight water deficit, water status (Fig. 7b). As in other semi-arid environments (Flores & Jurado 2003), soil nutrient content was higher (Fig. 2) and microclimatic conditions better (Fig. 3) under nurses than in gaps, which may be the main mechanism underlying facilitative effects of *Cistus* shrubs on understorey *Stipa* plants.

There is no evidence of negative effects of shrub shade on the short-term (i.e. photosynthetic rates) or long-term (biomass) carbon gain of young *Stipa* plants. Reduction of photosynthetic photon flux density (PFD) levels beneath the shrubs was low, and *Stipa* tussocks received more than 10 h day^{-1} of saturating PFD (data not shown). In this semi-arid region, *Stipa* plants are chronically photo-inhibited during summer owing to the combination of high irradiance, water deficit and high temperatures (Balaguer *et al.* 2002). Thus, shrub shade also reduced photo-inhibition of juvenile *Stipa*, as shown by the enhanced photochemical efficiency of PSII.

Studies of nurse plants in semi-arid environments rarely report the improvement of water availability beneath canopies resulting from facilitator-induced changes in soil physical properties. However, coexisting *Cistus* and *Stipa* increased soil organic matter (SOM) to a greater extent than each species separately, and tended to reduce bulk density compared with bare soils (Fig. 2). Pugnaire *et al.* (2004) have shown that SOM under different semi-arid shrub species was positively correlated with soil water content. SOM influences soil physical properties and water-holding capacity (Duchaufour 1995), and increases soil aggregate stability (Boix-Fayos *et al.* 2001). Indeed, Joffre & Rambal (1988) and Pugnaire *et al.* (1996b) showed that water-holding capacity is usually greater under shrubs than in gaps. Therefore, soils where both species grew in close association were able to retain more water than soils under isolated plants, which explains why juvenile *Stipa* tussocks growing in soils where both species were originally associated – with or without the shrub removed – had better water relations than isolated tussocks (Fig. 7b), because the root system of juvenile *Stipa* plants in both situations had similar distribution and depth (C. Armas, personal observation). Changes in soil and water-holding capacity also seem to be the main factors involved in the only positive effect gained by the shrub from its association with juvenile *Stipa* (improved tissue water content under mild water deficits, Fig. 7a).

Our results showed transient shifts in the balance of the interaction depending on seasonal water availability. During periods of low water availability, the facilitative effect of *Cistus* on juvenile *Stipa* was not evident (Fig. 7b), and the greater competitive ability of *Stipa* reduced water status in *Cistus* (Fig. 7a). These data agree with reports from other dry environments showing that

facilitative effects of woody species on their understorey occurred at the beginning of the growing season, i.e. when water was readily available (Holzapfel & Mahall 1999; Ludwig *et al.* 2004) or during rainy years, but shifted to neutral or competitive during severe water shortage (Tielbörger & Kadmon 2000; Ludwig *et al.* 2004). This is not in opposition to models hypothesizing that the importance of positive interactions should increase with greater abiotic stress (Bertness & Callaway 1994; Callaway & Walker 1997; Holmgren *et al.* 1997; Brooker & Callaghan 1998), because, for such effects to occur, water must be above a given threshold. In addition, these models refer to habitats and ecosystems and not to seasonal environmental changes within a given habitat. The observed temporal variability of plant interactions and the differences in the spatial patterns between life stages suggest that the balance between positive and negative interactions may easily shift in response to environmental variability in space and time (Tielbörger & Kadmon 2000; Schenk *et al.* 2003; Ludwig *et al.* 2004), highlighting the dynamic nature of plant–plant interactions in time, which may have important consequences on plant community structure.

INTERACTION BETWEEN ADULT PLANTS

As *Stipa* plants matured, the net balance of their interactions with *Cistus* changed, as did their spatial arrangement. A small proportion of adult plants of both species remained associated (26% of all adult *Stipa*, $\chi^2 = 21.16$, $P < 0.001$, and 37.8% of large *Cistus* shrubs, $\chi^2 = 5.8$, $P < 0.02$, Fig. 1B) and seemed to be unaffected by the presence of the other species under any abiotic conditions (Figs 4–7). This effect could be due either to niche differentiation by root segregation or to a compensation between the negative effects of competition and the positive effects of facilitation.

Soils under mature *Stipa* tussocks have higher nutrient content (Sánchez 1995; Puigdefábregas & Sánchez 1996; Maestre *et al.* 2003), higher infiltration rates (Cerdà 1997) and higher water-holding capacity (Maestre *et al.* 2001) than in gaps. *Cistus* shrubs growing with *Stipa* may benefit from such improved soil characteristics, while at the same time developing their root system (up to near 1 m from the main trunk, C. Armas, personal observation) in areas beyond the reach of *Stipa* roots. Overall, these results suggest that facilitation in semi-arid habitats is important, and that the neutral interaction balance between adult plants could be interpreted as a trade-off between the benefits of coexistence and the adverse effects of resource competition.

POPULATION DYNAMICS AND *STIPA* LIFE HISTORY

The analysis of population structure and spatial arrangements in this community suggests a strong control of *Stipa* on the population dynamics of both species. *Stipa* seedlings grow associated with *Cistus* but

may eventually out-compete the shrub, and this may be one of the main reasons why association between mature grasses and shrubs is uncommon. This displacement of the nurse plants by the facilitated species seems to be common in dry environments (Archer *et al.* 1988; Valiente-Banuet *et al.* 1991; Flores-Martínez *et al.* 1994). Other processes, however, may have contributed to configure the observed population structure, including self-thinning or the short life span of *Cistus* (< 20 years) compared with the long-lived *Stipa*. The low proportion of spatial associations between adult individuals of the two species suggests that the number of individuals of *Cistus*, a pioneer species, decreases with time, leaving a *Stipa*-dominated community, which is a predominant feature in the semi-arid landscapes of south-east Spain.

In conclusion, interactions between *Cistus* and *Stipa* include both positive and negative effects, which result in a balance that shifts in sign and intensity with time, and that strongly influences long-term population structure in this community. The spatial association between adult plants suggests that there are no net negative effects for either species, i.e that among the interactions that are likely to occur, positive effects counterbalance negatives effects. However, the effects of *Cistus* on juvenile *Stipa* were positive, and mainly caused by an improvement of microclimatic conditions and soil characteristics under the *Cistus* canopy. Our data suggest that the short-term balance of the interaction is very variable for each species, and can be positive or negative according to the fluctuation of environmental conditions. These data show that a pioneer species, *Cistus clusii*, facilitates the establishment and growth of *Stipa tenacissima*, driving population dynamics and succession in this semi-arid community.

Acknowledgements

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