

## Canopy vs. soil effects of shrubs facilitating tree seedlings in Mediterranean montane ecosystems

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### Abstract

**Question:** Is the facilitative effect of nurse shrubs on early recruitment of trees mediated by a ‘canopy effect’ (microclimate amelioration and protection from herbivores), a ‘soil effect’ (modification of soil properties), or both?

**Location:** Two successional montane shrublands at the Sierra Nevada Protected Area, SE Spain.

**Method:** Seedlings of *Quercus* and *Pinus* species were planted in four experimental treatments: (1) under shrubs; (2) in open interspaces without vegetation; (3) under shrubs where the canopies were removed; (4) in open interspaces but covering seedlings with branches, mimicking a shrub canopy.

**Results:** Both effects benefited seedling performance. However, microclimatic amelioration due to canopy shading had the strongest effect, which was particularly pronounced in the drier site. Below-ground, shrubs did not modify soil physical characteristics, organic matter, total N and P, or water content, but significantly increased available K, which has been shown to improve seedling water-use efficiency under drought conditions.

**Conclusions:** We propose that in Mediterranean montane ecosystems, characterised by a severe summer drought, pioneer shrubs represent a major safe site for tree early recruitment during secondary succession, improving seedling survival during summer by the modification of both the above- and below-ground environment.

**Keywords:** Nurse shrub; Positive interaction; Potassium; Removal experiment; Shade.

**Nomenclature:** Castroviejo et al. (1986-2001) for *Quercus* and *Pinus* species, and Molero-Mesa et al. (1992) for shrub species.

### Introduction

Facilitation of seedlings by adult plants underlies successful regeneration of many species under environmental stressful conditions (Niering et al. 1963; Flores & Jurado 2003), thereby playing a key role in shaping the structure of plant communities. This interaction is due to the habitat-modifying capacity of nurse plants,

which can alter its environment both above (‘canopy effect’) and below-ground (‘soil effect’) benefiting seedling establishment. The ‘canopy effect’ involves at least two components: microclimatic amelioration and protection from herbivores. Understorey microclimate is characterized by lower irradiance and air temperature, and consequently lower evapotranspiration demands, than open areas without vegetation (Franco & Nobel 1988; Valiente-Banuet & Ezcurra 1991; Callaway 1992; Maestre et al. 2003). On the other hand, canopies of benefactor species can avoid or diminish damage to beneficiary species by the possession of structures that repel prospective herbivores (e.g. thorns, spines), or just by physically hiding them (Wahl & Hay 1995; Hjältén & Price 1997; Rebollo et al. 2002). The ‘soil effect’ involves the modifications that vegetation produces on chemical, physical and biological soil properties. Many studies have shown that woody species can increase soil nutrients and organic matter because of the accumulation of litter beneath their canopies (e.g. Soriano & Sala 1986; Aguiar & Sala 1994), trapping of windblown particles (Escudero et al. 1985; DeSoyza et al. 1997), or symbiotic associations with N-fixing micro-organisms (Chapin et al. 1994; Alpert & Mooney 1996). The ‘canopy effect’ and the ‘soil effect’ are also interrelated. Lower radiation under nurse plants reduce soil temperature and evaporative water losses, whereas higher organic matter content improves soil water retention, thereby causing soil moisture and rates of litter decomposition to be higher under nurses than in open areas (Joffre & Rambal 1993; Cornejo et al. 1994; Aguilera et al. 1999).

The importance of nurse-plant interactions varies with the physical and biological environment, as well as the physiological and demographic traits of the interacting species (Bertness & Callaway 1994; Callaway & Walker 1997). Likewise, the relative importance of canopy effects vs. soil effects could vary strongly depending on the characteristics of the system and the species considered. However, most studies aimed at elucidating the mechanisms underlying plant facilitation have been conducted in ecosystems characterised

by a distinct patchy pattern, such as savannas and arid ecosystems (Scholes & Archer 1997; Aguiar & Sala 1999). In these systems, woody plants facilitate vegetation growing beneath their canopies (mainly herbaceous species) by ameliorating the physical environment and specially by increasing soil fertility (Jackson et al. 1990; Callaway et al. 1991; Belsky 1994; Pugnaire et al. 1996; Tirado 2003). Far fewer studies have examined mechanisms enabling nurse relationships in other ecosystems and, in particular, between woody species (but see Kitzberger et al. 2000 and Rudgers & Maron 2003).

Recent experiments have shown that pioneer shrubs facilitate tree seedling survival and growth during secondary succession in Mediterranean mountains (Gómez-Aparicio et al. 2004). However, the specific mechanisms involved and the extent to which they operate in different environments and for different interacting species are still unknown. Shade appears to be a necessary condition for seedling establishment of several Mediterranean woody species (Gómez 2004; Castro et al. 2004a; Gómez-Aparicio et al. 2005). In addition, the habitat structure, topography, and inherent soil variability of Mediterranean montane forests may be more complex than the two-phase configuration of savannas and arid ecosystems, possibly obscuring potential plant-induced changes in soil properties. Therefore, in these systems, the 'soil effect' of shrubs in facilitative interactions may be secondary to the 'canopy effect'. The main objective of this study was to determine the relative importance of canopy and soil effects in the facilitation of tree seedlings by pioneer shrubs. Specifically, our main questions were: 1. What is the net effect of pioneer shrubs on tree seedling survival and growth during secondary succession? 2. Can the relative importance of the canopy effect vs. the soil effect be elucidated? 3. Do the mechanisms of facilitation vary depending on the sites and species considered?

## Material and Methods

### Study sites

The study was conducted at two sites in the Sierra Nevada Protected Area (Granada, SE Spain). Sites were chosen because of their contrasting environmental characteristics, since although both are under post-fire secondary succession, one site is a Mediterranean high-mountain mesic system whereas the other is a medium-mountain xeric system. The first site is located at 1800-1850 m a.s.l. on the northwestern slope of Loma de Panaderos (Trevenque area, 37°5' N, 3°28' W). The soil parent material is colluvium and residuum derived dominantly from limestone, with slopes ranging from 5° to 15°.

The original *Pinus sylvestris* var. *nevadensis* forest was burned in 1983. Since then, the area has been recolonized by different shrub species, mainly *Salvia lavandulifolia* ssp. *vellerea* (*Lamiaceae*), an evergreen shrub that reaches a maximum of 50 cm high and 2 m in diameter. Inter-shrub spaces are ca. 1-3 m in diameter. The climate is subhumid Mediterranean, with a mean precipitation of 846 mm.a<sup>-1</sup> (1991-2002; data from a meteorological station at the Cortijuela Botanical Garden). Summers are hot and dry. The mean minimum temperature in the coldest month (January) is -0.9°C, the mean maximum of the hottest month (July) is 29.0 °C, and the mean annual temperature is 11.5 °C.

The second site is located at 1450 m a.s.l. on the southern slope of Loma de Lanjarón (36°56' N, 3°29' W). The soil parent material is colluvium and residuum derived from mica schist, with slopes ranging between 15° and 20°. It was formerly a *Pinus halepensis* afforestation that was burned in 1985. Currently, the site is occupied by an early successional community dominated by the perennial shrubs *Ulex parviflorus* (*Fabaceae*) and *Santolina canescens* (*Asteraceae*). Both species reach 50-100 cm mean height and 1-2 m diameter; individuals are separated by less than 2 m. The climate is dry Mediterranean (548 mm.a<sup>-1</sup>, 1991-2002; data from the Lanjarón Meteorological Station). The mean minimum temperature in the coldest month (January) is 3.3 °C, the mean maximum of the hottest month (July) is 29.4 °C, and the mean annual temperature is 15.1 °C. Ungulates at both study areas are mainly represented by Spanish ibex *Capra pyrenaica*.

### Experimental design

In February 2000 one experiment per site was carried out. The basic design consisted of planting one-year-old tree seedlings in four treatments: (1) under the canopy of shrub species (Shrub treatment); (2) in open interspaces without vegetation (Open treatment); (3) under shrubs where the canopies were removed by clipping the stems just above the ground (Clipped Canopy treatment), and (4) in open interspaces without vegetation but covering seedlings with the branches removed in the Clipped Canopy treatment, mimicking a shrub canopy (Mimicked Canopy treatment). In each site, we chose to plant seedlings of tree species that would naturally occupy the area, resulting in some differences in species planted. However, one common species (*Quercus ilex*) was planted at both sites as a between-site control. We used the most abundant shrub species in each site as nurse plants, choosing shrubs of similar morphology (short stature) in order to minimize inter-site variability due to different shrub identity. Seedlings of *Quercus ilex*, *Pinus nigra* ssp. *salzmannii* and *Pinus*

*sylvestris* var. *nevadensis* were planted in Loma de Panaderos, and *Salvia lavandulifolia* ssp. *vellerea* was used as the nurse plant. Experimental seedlings were distributed according to a randomized block-design in three 2000-m<sup>2</sup> plots separated by about 200 m. In Loma de Lanjarón, we planted *Quercus ilex* and *Pinus halepensis* seedlings in one 2000-m<sup>2</sup> plot, using *Ulex parviflorus* and *Santolina canescens* as nurse plants. In each plot, 50 seedlings per tree species and treatment were planted, a total of 1800 seedlings in Loma de Panaderos and 800 in Loma de Lanjarón. In all cases, planting holes were dug 40 cm deep with a mechanical auger having a 20-cm diameter bit, with no further cultivation around the plants. Seedlings were supplied by the Junta de Andalucía (Granada, Spain) and grown in nurseries under similar conditions.

#### *Abiotic characterization of experimental treatments*

Abiotic environmental conditions in each experimental treatment were characterized by measuring above- and below-ground microclimate, soil water content, and soil physical and chemical characteristics. Microclimatic conditions were measured with HOBO H8 sensors (Onset Computer Corporation, Massachusetts, USA) located in six sampling stations per microhabitat distributed randomly within each study area. They recorded air temperature ( $T_a$ ), soil temperature at 5 cm depth ( $T_s$ ), global radiation ( $R$ ) and relative humidity ( $RH$ ) every 15 minutes during three summer cloudless days.  $RH$  and  $T_a$  values were used to calculate the vapour pressure deficit (VPD) as an indicator of the atmospheric evapotranspiration demand (Pearcy et al. 1989). Volumetric soil water content was recorded using ThetaProbe sensors (Delta-T Devices Ltd., Cambridge, UK). Measurements were made 15-16 July 2000 in 15 sampling points per microhabitat at 20 cm depth, because seedlings are considered to have the majority of their roots in this upper soil layer (authors' pers. obs.). Although we made no abiotic measures in 2001, climatic data showed that both years had a total of summer precipitation (2 mm in 2000 and 10 mm in 2001) well below the average (47 mm average, 1991-2002). Thus, we can assume that abiotic conditions reported here represent extreme drought conditions in the study zones during the two years of the experiment.

To analyse the physical and chemical soil characteristics we sampled the upper 15 cm of the soil using a standard bucket auger. Ten soil samples were collected from each treatment, placed in plastic bags and transported to the laboratory. Air-dry soils were sieved and the < 2-mm fraction was analysed for routine chemical and physical properties (Anon. 1994) at the Food and Agriculture Laboratory of Granada. Soils in Open and Shrub treatments at both study sites were sampled and

analysed the first year of study, whereas soils in the Mimicked and Clipped Canopy treatments were sampled and analysed in the second year. Because there was unexplainable variation in some characteristics of soils sampled at different times and analysed in different batches we only used data from the Open and Shrub treatments for statistical comparisons. This irregularity nevertheless did not significantly affect the interpretation of our results (see Results and Discussion below).

#### *Seedling measurements*

Seedlings were counted twice a year between February 2000 and February 2002, once after summer (October) and once after winter (April). For each seedling we recorded: (1) survival, together with the most likely cause of mortality; (2) height, from the soil surface to the apical bud; and (3) herbivory damage, estimated as the percentage of browsed shoots in relation to the total number of shoots. We calculated the accumulated Relative Height Growth (RHG) during the whole study period as  $(\ln H_2 - \ln H_1) / (t_2 - t_1)$ , where  $H_2$  and  $H_1$  represents the final and initial heights, and  $t_2 - t_1$  the time elapsed between measurements.

#### *Data analysis*

Differences in cumulative seedling survival were analysed, for each study site, by Generalized Logit Models (Proc CATMOD, SAS; Anon. 2002) using the maximum likelihood as estimation method (Stokes et al. 1995). We included as independent variables the treatment, species, and block in Loma de Panaderos, and the treatment, species, and nurse plant in Loma de Lanjarón. Differences between levels of significant factors were explored by partial  $\chi^2$ -tests after correcting by Bonferroni adjustment at  $\alpha < 0.05$  (Cabin & Mitchell 2000). To examine between-treatment differences in seedling RHG, we used General Linear Models (Proc GLM, SAS, Anon. 2002). One-way ANOVAs were performed for each combination of seedling species and study site, considering only treatments with more than one surviving seedling at the end of the experiment (i.e. Clipped canopy was excluded for *P. nigra* and *P. sylvestris* in Loma de Panaderos, and Clipped canopy and Open for *Q. ilex* in Loma de Lanjarón). We did not analyse differences in RHG among blocks or nurse plants due to the low number of surviving seedlings.

Between-treatment differences in abiotic conditions were analysed using one-way ANOVAs (Proc GLM, SAS 2002). For air temperature ( $T_a$ ), vapor pressure deficit (VPD), global radiation ( $R$ ) and soil temperature ( $T_s$ ) we considered as dependent variables the mean values recorded per sample point throughout the three

days, in order to avoid pseudoreplication. Data used in the ANOVAs were tested for normality and homoscedasticity and transformed when necessary. Throughout this paper, means are shown  $\pm 1$  SE.

## Results

### Abiotic characterization of experimental treatments

All the microclimatic variables differed significantly among treatments at both study sites (Table 1). Mean air temperature, radiation and vapour pressure deficit were in all cases higher in Open and Clipped Canopy than in Shrubs and Mimicked Canopy treatments (Table 1). Soil temperature was also lower in Shrubs and Mimicked Canopy than in the other two treatments (Table 1). However, there were no differences in soil water content among microhabitats, with values ranging from 8-10% in Loma de Panaderos and 1-3% in Loma de Lanjarón (Table 1).

In relation to soil chemical and physical properties, treatments differed significantly only in pH and available K (Table 2). The pH was higher in the Shrub ( $7.58 \pm 0.08$ ) than in the Open treatment ( $7.19 \pm 0.04$ ) in Loma de Panaderos, although differences were relatively small, both pH values being very slightly alkaline. Available K was also much higher under *S. lavandulifolia* than in Open ( $F = 14.25$ ,  $df = 1;8$ ,  $p = 0.001$ ). The same pattern was found in Loma de Lanjarón, where available K under *U. parviflorus* and, especially, under *S. canescens* was higher than in the Open treatment ( $F = 7.81$ ,  $df = 1;8$ ,  $p = 0.012$  and  $F =$

18.91,  $df = 1;8$ ,  $p = 0.0004$ , respectively).

### Seedling measurements

Of the initial seedlings planted, only 21.9% survived in Loma de Panaderos and 13.8% in Loma de Lanjarón until the end of the experiment. About 90% of mortality occurred during the first summer at both study sites, mainly due to summer drought (Fig. 1). Cumulative seedling survival differed among treatments in the two study sites (Table 3). The highest survival occurred in the Shrub and the lowest in the Open treatment, survival increasing by a factor of two in Loma de Panaderos (39.3% in Shrub vs. 18.9% in Open) and by more than an order of magnitude in Loma de Lanjarón (25.6% in Shrub vs. 2.0% in Open). Survival in the Mimicked Canopy and Clipped Canopy treatments was generally intermediate between the Open and Shrub treatments (Fig. 1).

Survival differed significantly between tree species at both study sites (Table 3). In Loma de Panaderos, survival of *Q. ilex* seedlings (48.0%) was over four times higher than *P. nigra* survival (10.9%) and more than eight times higher than *P. sylvestris* survival (5.7%). In Loma de Lanjarón, *P. halepensis* survival (19.2%) was more than two times higher than *Q. ilex* survival (8.2%). When compared across sites, *Q. ilex* survival was much higher in moister Loma de Panaderos than in Loma de Lanjarón. Furthermore, the increment in survival caused by shrubs in comparison to Open was much higher in drier Loma de Lanjarón (from 0% to 20.5%) than in Loma de Panaderos (from 25.2% to 70.9%; Fig. 1).

**Table 1.** Microclimatic conditions ( $n = 6$ ) and soil volumetric water content ( $n = 10$ ) measured in the experimental treatments (means  $\pm 1$  SE). Radiance is expressed as percentages of radiance in the Open treatment. The results of the tests for Loma de Panaderos are:  $F = 26.97$ ,  $df = 3;20$ ,  $p < 0.0001$  for  $T_a$ ;  $F = 47.45$ ,  $df = 3;20$ ,  $p < 0.0001$  for  $R$ ;  $F = 36.57$ ,  $df = 3;20$ ,  $p < 0.0001$  for VPD;  $F = 18.17$ ,  $df = 3;20$ ,  $p < 0.0001$  for  $T_s$ ; and  $F = 1.57$ ,  $df = 3;36$ ,  $p = 0.21$  for VWC. The results for Loma de Lanjarón are:  $F = 13.94$ ,  $df = 6;35$ ,  $p < 0.0001$  for  $T_a$ ;  $F = 14.01$ ,  $df = 6;35$ ,  $p < 0.0001$  for  $R$ ;  $F = 13.67$ ,  $df = 6;35$ ,  $p < 0.0001$  for VPD;  $F = 11.89$ ,  $df = 6;35$ ,  $p < 0.0001$  for  $T_s$ ; and  $F = 1.52$ ,  $df = 6;63$ ,  $p = 0.18$  for VWC. Different letters show significant differences at  $\alpha < 0.05$  (after Bonferroni correction) for the same variable and study site.

Treatment	$T_a$ (°C)	Above-ground $R$ (%)	VPD (MPa)	Below-ground	
				$T_s$ (°C)	VWC (%)
<b>Loma de Panaderos</b>					
<i>Salvia lavandulifolia</i>	29.51 $\pm$ 0.31a	57.1 $\pm$ 0.98a	2.94 $\pm$ 0.04a	23.3 $\pm$ 0.21a	9.31 $\pm$ 0.40a
<i>Salvia lavandulifolia</i> Mimicked Canopy	32.73 $\pm$ 0.35b	69.7 $\pm$ 2.56b	3.34 $\pm$ 0.05b	29.9 $\pm$ 0.22b	8.30 $\pm$ 0.22a
<i>Salvia lavandulifolia</i> Clipped Canopy	39.51 $\pm$ 0.62c	94.8 $\pm$ 0.44c	6.02 $\pm$ 0.22c	28.8 $\pm$ 0.30c	8.48 $\pm$ 0.34a
Open	38.73 $\pm$ 0.51c	99.1 $\pm$ 0.64c	5.78 $\pm$ 0.14c	32.4 $\pm$ 0.33d	8.73 $\pm$ 0.47a
<b>Loma de Lanjarón</b>					
<i>Ulex parviflorus</i>	30.67 $\pm$ 0.21a	38.7 $\pm$ 0.78a	2.79 $\pm$ 0.05a	28.1 $\pm$ 0.25a	1.92 $\pm$ 0.43a
<i>Santolina canescens</i>	32.34 $\pm$ 0.42a	62.2 $\pm$ 0.99b	3.24 $\pm$ 0.08b	29.8 $\pm$ 0.35a	1.71 $\pm$ 0.34a
<i>Ulex parviflorus</i> Mimicked Canopy	35.07 $\pm$ 0.55ab	50.8 $\pm$ 2.56c	3.06 $\pm$ 0.11ab	32.8 $\pm$ 0.31b	1.81 $\pm$ 0.21a
<i>Santolina canescens</i> Mimicked Canopy	36.31 $\pm$ 0.54b	72.8 $\pm$ 2.72d	4.33 $\pm$ 0.12c	33.0 $\pm$ 0.40b	1.37 $\pm$ 0.20a
<i>Ulex parviflorus</i> Clipped Canopy	43.46 $\pm$ 0.94c	96.6 $\pm$ 0.78e	7.04 $\pm$ 0.38d	35.9 $\pm$ 0.50c	1.18 $\pm$ 0.33a
<i>Santolina canescens</i> Clipped Canopy	43.11 $\pm$ 0.95c	95.6 $\pm$ 0.54e	6.91 $\pm$ 0.36d	36.9 $\pm$ 0.63c	1.95 $\pm$ 0.36a
Open	42.72 $\pm$ 0.77c	99.3 $\pm$ 0.45e	5.94 $\pm$ 0.24d	35.6 $\pm$ 0.51c	2.39 $\pm$ 0.32a



**Table 2.** Summary of soil physical and chemical characteristics in Shrub and Open treatments. For each study site, variables that significantly differed between treatments at  $\alpha < 0.05$  (after Bonferroni correction) are shown in bold.

Variable*	Loma de Panaderos		Open	Loma de Lanjarón	
	Open	<i>Salvia lavandulifolia</i>		<i>Ulex parviflorus</i>	<i>Santolina canescens</i>
Clay (%)	30.72 ± 1.02	31.02 ± 1.01	7.40 ± 0.27	8.50 ± 0.31	7.90 ± 0.31
Sand (%)	23.43 ± 2.71	19.92 ± 0.62	64.60 ± 1.03	64.91 ± 0.69	65.00 ± 0.54
AWC (%)	18.19 ± 1.94	23.98 ± 0.89	11.67 ± 0.45	12.26 ± 0.52	12.66 ± 0.49
pH	<b>7.19 ± 0.04</b>	<b>7.58 ± 0.08</b>	6.55 ± 0.09	6.74 ± 0.08	6.87 ± 0.06
CEC (cmol <sub>c</sub> /kg)	19.12 ± 1.38	17.49 ± 0.67	8.87 ± 0.47	9.50 ± 0.36	9.71 ± 0.26
Ca (cmol <sub>c</sub> /kg)	15.39 ± 0.93	13.54 ± 0.59	7.25 ± 0.42	7.24 ± 0.43	7.77 ± 0.28
OM (%)	4.84 ± 0.47	5.46 ± 0.33	1.62 ± 0.32	2.11 ± 0.17	1.77 ± 0.14
N (%)	0.29 ± 0.02	0.31 ± 0.02	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
P (mg/kg)	4.10 ± 0.41	6.10 ± 0.78	1.80 ± 0.39	1.40 ± 0.16	1.40 ± 0.22
K (mg/kg)	<b>138.00 ± 10.52</b>	<b>204.05 ± 15.36</b>	<b>88.00 ± 10.91</b>	<b>127.00 ± 11.03</b>	<b>183.50 ± 21.49</b>

\* Soil variables were determined as follows: clay and sand by the Bouyoucos hydrometer method; available water capacity (AWC) as the difference in the amount of soil water between field capacity (pf 1/3) and permanent wilting point (pf 15) in a pressure chamber; pH with a pH meter in a 1:2.5 mass:volume soil and water suspension; cation exchange capacity (CEC) and exchangeable Ca by removal of soluble fraction, extraction with ammonium acetate and concentrations measures using atomic emission spectroscopy; organic matter (OM) by loss of mass by combustion at 430°C; total N by the Kjeldahl method; available P by colorimetry with bicarbonate of soda; and available K (soluble plus exchangeable) by extraction with ammonium acetate and atomic emission spectroscopy.

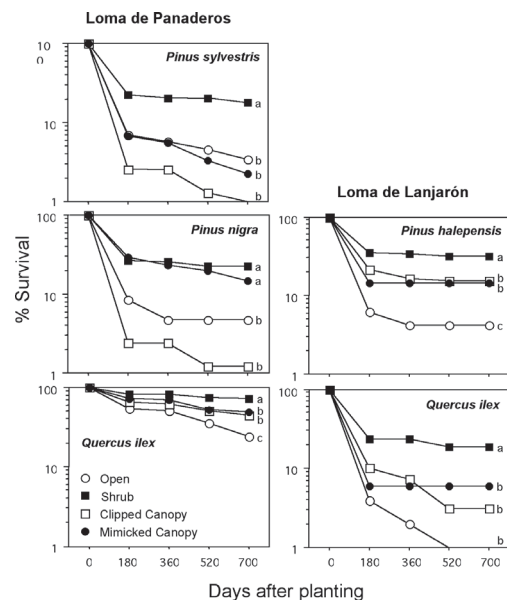
There were significant differences in the response of tree species to treatments in Loma de Panaderos (significant Treatment × Species interaction term; Table 3) but not in Loma de Lanjarón. Thus, survival of *Q. ilex* in Loma de Panaderos was boosted by the Mimicked and the Clipped Canopy treatments, survival of *P. nigra* only by the Mimicked Canopy treatment, and survival of *P. sylvestris* by none of the treatments (Fig. 1). Finally, there were significant between-block differences in seedling survival in Loma de Panaderos (Table 3). No between-nurse plant differences in seedling survival appeared in Loma de Lanjarón (Table 3).

RHG was very low in all cases, not surpassing 0.6 mm/year for any species and treatment combination. It differed between treatments only for *Q. ilex* in Loma de Panaderos ( $F = 4.946$ ,  $df = 3$ ,  $p = 0.003$ ), being higher in

Shrub ( $0.19 \pm 0.01$  mm.a<sup>-1</sup>) and Mimicked Canopy ( $0.09 \pm 0.01$  mm.a<sup>-1</sup>) than in the Clipped Canopy ( $0.04 \pm 0.02$  mm.a<sup>-1</sup>) and the Open ( $0.04 \pm 0.01$  mm.a<sup>-1</sup>) treatments. The percentage of seedlings damaged by ungulates was lower than 4% in all study years and sites, the damage being homogeneously distributed between treatments.

**Table 3.** Summary of the contingency analysis for seedling survival at the end of the experiment.

Study site /Factor	df	$\chi^2$	p
<b>Loma de Panaderos</b>			
Treatment	3	71.68	< 0.0001
Species	2	224.92	< 0.0001
Block	2	25.34	< 0.0001
Treatment × Species	6	18.03	0.0062
Treatment × Block	6	9.29	0.1577
Species × Block	4	6.39	0.1717
Model	23	351.05	< 0.0001
<b>Loma de Lanjarón</b>			
Treatment	3	43.27	< 0.0001
Species	1	11.11	0.0009
Nurse	1	0.55	0.4568
Treatment × Species	3	8.50	0.2479
Treatment × Nurse	3	1.71	0.6353
Species × Nurse	3	1.43	0.2315
Model	12	62.89	< 0.0001



**Fig. 1.** Survival of tree seedlings planted in the four experimental treatments. Different letters show significant differences at  $\alpha < 0.05$  (after Bonferroni correction) in accumulated survival at the end of the experiment.

## Discussion

### *Net effect of shrubs over seedling performance*

Our results have shown that pioneer shrubs facilitate early recruitment of tree seedlings in the Mediterranean mountains, thereby showing the relevance that positive interactions play during secondary succession in these type of ecosystems (see also Maestre et al. 2001, 2003; Castro et al. 2004b; Gómez-Aparicio et al. 2004). Furthermore, the higher positive effect of shrubs in Loma de Lanjarón than in Loma de Panaderos (both when considering all tree species pooled and just for *Q. ilex*) agrees with the conceptual model that predicts the importance of positive interactions to increase with abiotic stress (Bertness & Callaway 1994; Holmgren et al. 1997). Thus, in Loma de Lanjarón, the lower altitude, southern aspect, steeper slope, coarser soil textures and lower available water capacity could have all contributed to greater drought stress than at Loma de Panaderos, resulting in higher seedling mortality during the critical first summer. Between-site differences in the positive effect of shrubs could also have been influenced by differences in the species of the nurse plants. However, as shrubs at both sites had similar effects over soil and microclimatic characteristics, it is likely that inter-site differences were mainly derived from their contrasting abiotic conditions. Differences in facilitation importance in relation to stress level were also found between tree species in the same study site. The higher the percentage of total mortality for a tree species, the more shrubs boosted survival when compared to open areas. Therefore, both inter- and intra-sites differences in the effect of shrubs on survival were highly linked both to the 'absolute' stress level of the system (or stress measured in abiotic terms) and to the 'functional' stress level (or stress measured as species-specific responses).

### *Relative importance of soil vs. canopy effects*

Seedling survival was higher in Shrub than in any other treatment at both study sites, whereas survival in the Mimicked and Clipped Canopy treatments was generally intermediate. These findings suggest that there was not a net competitive effect, neither above- nor below-ground, between shrubs and tree seedlings. On the contrary, both the canopy effect and the soil effect were positive and thereby responsible for the facilitative effect that shrubs had on tree seedlings (see Carrillo-García et al. 2000 for a similar result). Moreover, both effects together were necessary to improve seedling survival in cases of high 'functional' stress levels. Hence, when seedling mortality was greater than or equal to 90%, as was the case for *P. sylvestris* in Loma de

Panaderos and *Q. ilex* in Loma de Lanjarón, only whole shrubs functioned as safe sites for recruitment.

Nevertheless, our canopy manipulation experiments suggested that the modification of soil physical and chemical properties by shrubs (soil effect) exerted a lower benefit over seedling survival and growth than the canopy effect. In fact, at both study sites seedling survival was generally lower when shrub canopies were removed (Clipped Canopy treatment) than when the influence of shrubs on soil properties was 'eliminated' (Mimicked Canopy treatment). This result agrees with the limited effect of nurse plants on soil properties found in this study. The presence of pioneer shrubs did not modify soil physical characteristics such as texture or available water capacity, nor increase organic matter accumulation. Soil nutrient capacity (CEC, N and P) was also similar, despite one of the nurse plants considered, *Ulex parviflorus*, was a nitrogen-fixing legume. In general, soil enrichment by legumes has been shown to have beneficial effects on neighbours in nutrient-poor environments (see Shumway 2000 and references therein), as is the case of Mediterranean systems (Gallardo 2001). However, *Ulex parviflorus* shows a low rate of N-fixation, together with an important accumulation of N in standing necromass (Huesca et al. 1998), which could help its minimal effect on soil fertility. The absence of a stronger soil effect of pioneer shrubs, in contrast to results reported for other late-successional systems like savannas, can be caused by several non-exclusive characteristics of the Mediterranean systems studied, such as (1) a higher density of shrubs, which determines a lower capacity of resource nucleation per nurse shrub; (2) their lower age and size, which implies less time to affect abiotic conditions; and (3) lower inter-patch distance, which diminishes probabilities of finding below-ground open spaces out of reach of any root system.

However, nurse shrubs effectively modified one soil property, available K, being in all cases higher under shrubs than in open interspaces. Potassium seems to increase plant resistance to drought, mainly due to osmotic adjustments and a reduction in transpiration rates, which in turn involves a higher water-use efficiency (Bradbury & Malcom 1977; van den Driessche 1991; Egilla et al. 2001). Despite its potential benefit, this nutrient has never been highlighted when analysing facilitation mechanisms by nurse plants, with most studies focusing on the modification of soil nitrogen as the main nutrient-related facilitation mechanism (Pugnaire et al. 1996; Moro et al. 1997; Carrillo-García et al. 2000). In this sense, the increase in soil potassium caused by shrubs could be an overlooked but relevant soil effect on facilitation in systems where summer drought constitutes the main limiting factor for plant survival in the field.

The canopy effect due to microclimate amelioration was the main mechanism enhancing seedling survival and growth (see also Valiente-Banuet & Ezcurra 1991; Valiente-Banuet et al. 1991; Greenlee & Callaway 1996; and Maestre et al. 2001). Lower air and soil temperatures, radiation and vapor pressure deficit measured below shrub canopies in comparison to open areas reduced leaf temperature and transpiration losses, consequently inducing a more favourable water balance in tree seedlings. This array of modifications could be critical for seedlings to withstand the summer drought, consequently suggesting that shade constitutes a key facilitative mechanism in Mediterranean systems, even without improving soil water conditions (as it occurred in our study; Holmgren et al. 1997; Domingo et al. 1999). Protection against herbivores, another potential benefit of being covered by shrubs, could not be explored, since ungulates injured very few seedlings presumably due to both the small sapling height (and consequent difficulty in being found) and the low ungulate densities in the study areas.

To summarize, we proposed that, although canopy shading appeared as the main factor involved in the interaction under study, modification of potassium content by shrubs could also contribute to enhanced tree seedling survival during the dry and hot Mediterranean summers. Therefore, in scenarios where drought constitutes the main factor limiting secondary succession, pioneer shrubs can represent the only safe sites for woody species recruitment thanks to their widespread capacity of habitat modification both above- and below-ground.

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