

Nurse-plant effects on the seed biology and germination of desert annuals

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Abstract Nurse-plants generally have positive effects on understorey species by creating more suitable conditions for stress-intolerant plants relative to open micro-habitats. However, long-term effects of this plant–plant facilitation system have been rarely examined. Seeds of five desert annual species from Atiquipa coastal desert in Southern Peru were used to examine whether different microenvironmental conditions under the nurse-plants *Caesalpinia spinosa* Molina (Kuntze) lead to differences in seed biology and germinability of annual plants relative to open, canopy-free conditions. Seeds collected from plants associated with nurse-plants were predicted to be (i) larger due to more favourable growing conditions, (ii) more viable and with greater germination rates, (iii) less variable in size and viability due to reduced environmental heterogeneity, and (iv) to germinate faster to avoid apparent competition with other annuals. Seed attribute measurements and germination trials in growth chambers were used to test these predictions. Although the plant abundance of only 2 of 5 species was strongly facilitated by the nurse-plant, no significant differences were found in seed mass, viability or relative variability between understorey and open micro-habitats for any of the species. Contrary to our predictions, final seed germination rates of seeds from open micro-habitats were higher, and the open micro-habitat treatment was more favourable for germination of seeds from both open and understorey environments. Taken together, these results suggest that plant–plant facilitation does not necessarily affect seed biology traits. Further studies addressing larger distribution ranges and/or density gradients of understorey species will illuminate the potential evolutionary effects of nurse-plants.

Key words: differentiation, ecotypes, facilitation, positive effects, viability.

INTRODUCTION

Consequences of positive effects in plant communities (i.e. facilitation) have been widely demonstrated and incorporated into general ecological theory (Bruno *et al.* 2003; Callaway 2007; Brooker *et al.* 2008). Facilitation effects on the frequency of occurrence of species have been shown to occur in several ecosystems, although most frequently in stressful environments such as deserts (Bertness & Callaway 1994; Flores & Jurado 2003; Holzapfel *et al.* 2006). Nurse-plants have been championed by ecologists as clear examples of positive interactions in stressful environments (Maestre *et al.* 2003; Gomez-Aparicio *et al.* 2004). Effects of nurse-plants have usually been described in terms of an increase in abundance and/or diversity of understorey plants compared with neighbouring open environments, that is, away from the nurses (Pugnaire *et al.* 1996; Holzapfel *et al.* 2006). Nurse-plants generate more benign habitats that allow stress-intolerant species to persist under extreme environmental conditions (Liancourt *et al.* 2005; Maestre

et al. 2009). Facilitation at local scales is likely more frequent in ecosystems subject to significant perturbation or abiotic limitation (Kéfi *et al.* 2008). Local facilitation depends on low dispersal ability of the target species, and this is a trait generally reported for desert annuals (Ellner & Shmida 1981; Venable *et al.* 2008; Ward 2009). Venable *et al.* (2008) demonstrated that the mean dispersal distances for these species are on average relatively small at less than 1 m. Wilson (1993) showed that the mean dispersal distance was 0.92 m for herbaceous species with morphological adaptations for wind dispersal, and 0.49 m for herbaceous species with no apparent dispersal mechanism. Moreover, nurse-plants usually act as seed traps and barriers for the dispersal of understorey species (Bullock & Moy 2004; Giladi *et al.* 2013). Low dispersal ability in stressful environments could potentially lead to ecotypic differentiation between plants from understorey and open micro-habitats. This hypothesis has however remained unexamined to date (but see Liancourt & Tielbörger 2011). Moreover, increased abundance of plants under nurse-plants can also lead to increased competition (or apparent competition), although evidence of this is equivocal to date (Tielbörger & Kadmon 2000; Seifan *et al.* 2010).

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Increased competition among understorey plant species associated with nurse-plants (i.e. apparent competition mediated by the nurse-plant by increasing negative effects among understorey plant species competing for abiotic resources) could in theory induce accelerated adaptive germination (Dyer *et al.* 2000; Goldberg *et al.* 2001) to avoid such competitive effects. Ecotypic differentiation and local adaptation following nurse-plant facilitation are therefore two important evolutionary processes that may occur in harsh environments. They nonetheless represent critical research gaps in plant interaction ecology (Callaway 2007; Brooker *et al.* 2008; Thorpe *et al.* 2011), and a close examination of changes in life-history traits of understorey annual plants is a pertinent starting point to address them.

Desert plant species cope with harsh conditions by avoiding them (i.e. annuals) or enduring them (i.e. shrubs) (Whitford 2002; Ward 2009). For annuals, seed production represents their only link between one generation and the next, and seed germination is also critical (Pake & Venable 1996; Facelli *et al.* 2005). Germination of annuals has to be finely tuned with environmental cues to ensure that germinating plants will produce new seeds for the species to persist (Noy-Meir 1973; Venable 2007). To this end, many strategies have been described such as dormancy to remain latent until appropriate environmental conditions arise (Baskin & Baskin 2001), bet-hedging in which plants delay short-term germination in favour of long-term fitness increases (Venable & Lawlor 1980), or age structuring of seed banks (Chesson 2000; Adonakis & Venable 2004). However, in the context of nurse-plants, little is known on whether the positive effects experienced by plants in the understorey translate into increased seed germination capabilities due to improved micro-environmental conditions, and whether these traits would be evolutionary stable (Thorpe *et al.* 2011). Maternal effects expressed in seeds traits are commonly studied and well established (Roach & Wulff 1987; Galloway 2005; Donohue 2009). Increased size (Sultan 1996; Valencia-Díaz & Montaña 2005), germination fraction and viability (Baskin & Baskin 2001; Valencia-Díaz & Montaña 2005; Breen & Richards 2008) of seeds have been reported as consequences of the maternal plant experiences including environmental conditions. However, few studies track the effects of facilitation to the seeds (but see Liancourt & Tielbörger 2011), even for more evident facilitation consequences on seeds such as seed traps (Pugnaire & Lazaro 2000). Given that nurse-plants commonly facilitate annuals and that the seed bank is a critical tool for persistence, nurse-plant effects on seeds thus represent a critical question to explore longer-lasting consequences of plant facilitation such as ecotypic differentiation.

The purpose of this study was to determine the effects of nurse plants on seed biology and germination of understorey annual plant species relative to the same species growing in open micro-habitats. We hypothesized that facilitation by nurse-plants generates sufficiently different micro-environmental conditions that lead to consistent differences in seeds traits of understorey plants. We explored the following predictions to test this hypothesis: seeds collected from plants associated with the nurse-plant micro-habitat would (i) be larger due to more favourable growing conditions, (ii) have greater viability and germination rate (iii) have less variability in size and viability due to reduced environmental heterogeneity provided by nurses (buffering), and (iv) germinate faster due to potential apparent competition with other annuals. While many studies have examined and documented the importance of nurse plant-plant interactions for understorey plant diversity and abundance (Callaway 2007; Brooker *et al.* 2008), few of them have explored the potential evolutionary implications for beneficiary species by examining other life-history stages such as seed viability and germination (but see Liancourt & Tielbörger 2011). Reciprocal common gardens are an important approach to study trait sets under sets of conditions that a species may be associated with, particularly when the habitats are very discrete (Hufford & Mazer 2003; Maron *et al.* 2004). A smaller-scale version of this approach is applied here using a reciprocal germination design in growth chambers programmed to emulate each set of conditions from field measurements (open versus understorey). This design identifies whether there is preliminary evidence for ecotypic differentiation in seed traits driven by nurse-plant facilitation.

METHODS

Study site and species

Seeds of five annual species were collected from Atiquipa, Southern Peru (15°S, 74°W). Atiquipa is a coastal desert with a wet winter season occurring between July and November and characterized by high moisture due to fog (about 90%) and with about 70% of the annual rainfall mean (e.g. 200 mm) falling between those months (Sotomayor & Jimenez 2008). One of the nurse-plant species of this location is the locally abundant 4–5 m tall tree *Caesalpinia spinosa* Molina (Kuntze) (Fabaceae). This tree is native to Peru, where is abundant, but it can also be found in various places of South America such as Bolivia, Colombia, Ecuador and Venezuela tolerating dry climates and poor soils (Sprague 1931). The five understorey annual species used in this study were selected because of their relatively high abundance and their contrasting relative patterns of abundance from high to low densities in the understorey of nurse-plants. They were *Alonsoa meridionalis* (L. f.) Kuntze (Scrophulariaceae),

Cyperus hermaphroditus (Jacq.) Standl. (Cyperaceae), *Fuertesimalva peruviana* (L.) Fryxell (Malvaceae), *Nassella mucronata* (Kunth) R.W. Pohl (Poaceae), and *Plantago limensis* Pers (Plantaginaceae). Both *Cyperus hermaphroditus* and *Nassella mucronata* correspond to grass-like species that grow up to 30–40 cm tall and produce 80–150 seeds per plant. Meanwhile, *Alonsoa meridionalis* and *Fuertesimalva peruviana* can grow up to the size of little shrubs (about 80 cm tall) and produce thousands of seeds from their numerous flowers (especially for *Alonsoa meridionalis*). *Plantago limensis* is a small annual rosette plant that grows up to 20–30 cm and produces 40–70 seeds per individual. All 5 species are distributed along the Andes from 0 to about 4000 m asl in South America, although *Plantago limensis* is endemic to Peru (Brako & Zarucchi 1993).

Entire plants including their seeds were collected in December 2012 (end of spring/start of summer) across five sites separated by about 2 km within Atiquipa, right after seed set and plant senescence. Both understorey (i.e. under nurse-plant) and open micro-habitats were sampled in each site, where plants were harvested in pairs with one individual from understorey and one from open micro-habitat. Pairs of plants were carefully selected to span the whole species distribution range in each location, and were therefore at least 5 m apart to ensure the collection of different meta-populations considering average dispersal distances reported in the literature (see Wilson 1993; Venable *et al.* 2008). At least five pairs of plants (i.e. replicates) were collected in each site for each species, for a total of 125 pairs, that is 250 plants.

Plant density

Plant density of each species in each micro-habitat was recorded at the peak of the 2012 growing season (October) using 0.25 m² quadrats ($n = 90$): 9 quadrats per micro-habitat type paired across the five locations used for seed collection. The strength of nurse-plant effect on each understorey plant species density was reported via the Relative Interaction Index (RII), which was calculated as follows (Armas *et al.* 2004):

$$RII = \frac{D_u - D_o}{D_u + D_o} \quad (1)$$

The terms D_u and D_o corresponded to the density of plants in understorey and open micro-habitats, respectively. This index varies from -1 to $+1$ with positive effects being >0 and negative effects <0 on the density of these species.

Seed mass and viability

Because of their small size, seeds of all species were weighed in groups of 10 using a Mettler Toledo MX5 scale (0.1 µg precision). A total of 200 seeds were weighed per micro-habitat per species. Seed viability was assessed via tetrazolium (Tz) tests, a valid method for both dormant and non-dormant embryos (Flemion & Poole 1948; Baskin & Baskin 2001). Embryos were dissected from 24-h water-imbibed seeds, and then placed in a 1% solution of 2,3,5-

triphenyl-2H-tetrazolium chloride (TTC) for another 24 h before evaluation. They were considered viable when they had turned red or pink due to the reaction between TTC and hydrogen ions released by embryos during respiration (Baskin & Baskin 2001). This test was carried out on 25 randomly selected seeds per replicate, with 4 replicates per micro-habitat per species (200 seeds per species, 100 per micro-habitat).

Seed germination trials

Germination trials were conducted in growth chambers whose conditions simulated both open and understorey micro-habitats. Micro-habitat conditions were measured *in situ* using HOBO U-23 Pro-V2 loggers for temperature and humidity (two in each micro-habitat, understorey of *Caesalpinia spinosa*), and HOBO UA-002–64 loggers for light intensity and temperature (one in each micro-habitat). Loggers were installed in the field site on August 2011, and recorded micro-habitat conditions during the whole growing season, that is until mid-October 2011. Data from the loggers were aggregated to obtain 2-h means for temperature, relative humidity and light intensity in order to program the growth chambers simulating our field site conditions (Table 1). Mean temperature and relative humidity were best simulated in the growth chambers; however, light conditions, especially for the open maximum, did diverge from field site conditions (Table 1). Overall, the growth chambers were able to effectively simulate the general patterns of relative differences between open and understorey micro-habitats, that is warmer, less humid and with increased illuminance towards the middle of the day for open micro-habitats.

The growth chamber experiment was run in six growth chambers (Sanyo MLR-351H, Japan), with three of them simulating understorey micro-habitat conditions, and three simulating open space micro-habitat conditions. We utilized a full-factorial reciprocal design using 10 seeds per replicate and the following factors: 2 germination environments (open, understorey), 2 seed sources (open, understorey), and 5 species, with 10 replicates per treatment. This was a total of 200 experimental units tested. Seed germination was recorded every 2–3 days until no further changes were observed for at least one week (after 27 days in total). A seed was considered germinated when the radicle or coleoptile was visible by 1 to 2 mm.

Statistical analyses

Generalized linear mixed models (GLMMs) were used to test for differences in plant density, seed mass and seed viability among species and seed sources, including the species \times source interaction. In order to test for differences in relative scaled variability between sources, we calculated coefficients of variation (CVs) for seed mass and seed viability estimates, and compared those using *t*-tests with species as replicates. Germination trial responses were condensed into final germination rate and number of days to 50% germination. These variables were also analysed with GLMMs, using simulated micro-habitat, seed source, species

Table 1. Micro-habitat data for open (O) and understorey (U) conditions obtained from field observations at Atiquipa, Southern Peru

Hour of the day	Temperature (°C)		Relative humidity (%)		Illuminance (lx) [†]	
	O	U	O	U	O	U
0–4	12.5 ± 0.1 (12.5)	12.2 ± 0.1 (12.2)	84.5 ± 0.6 (85)	86.1 ± 0.4 (86)	0.0 ± 0 (0)	0.0 ± 0 (0)
4–8	11.8 ± 0.1 (11.8)	11.7 ± 0.1 (11.7)	85.1 ± 0.6 (85)	86.6 ± 0.4 (87)	93.9 ± 10.5 (0)	68.1 ± 20.8 (0)
8–10	12.5 ± 0.1 (12.5)	12.6 ± 0.2 (12.6)	86.0 ± 0.9 (86)	87.1 ± 0.7 (87)	2860.7 ± 150.7 (2)	6402.3 ± 1048.7 (3)
10–12	15.0 ± 0.2 (15.0)	14.2 ± 0.2 (14.2)	84.1 ± 0.9 (84)	87.3 ± 0.7 (87)	10862.0 ± 594.9 (4)	4005.6 ± 458.5 (2)
12–14	22.4 ± 0.3 (22.4)	15.2 ± 0.2 (15.2)	76.9 ± 0.9 (77)	86.7 ± 0.7 (87)	54855.2 ± 2412.4 (5)	1247.3 ± 30.6 (1)
14–16	24.9 ± 0.4 (24.9)	15.1 ± 0.2 (15.1)	72.2 ± 1.0 (72)	86.2 ± 0.7 (86)	59898.1 ± 2629.5 (5)	866.5 ± 19.2 (1)
16–18	20.8 ± 0.3 (20.8)	14.5 ± 0.2 (14.5)	75.4 ± 1.0 (75)	85.7 ± 0.7 (86)	19444.1 ± 1120.2 (4)	373.5 ± 17.0 (1)
18–20	15.3 ± 0.2 (15.3)	13.3 ± 0.1 (13.3)	82.7 ± 0.9 (83)	86.0 ± 0.6 (86)	993.9 ± 128.3 (1)	27.3 ± 2.7 (0)
20–24	13.4 ± 0.1 (13.4)	12.6 ± 0.1 (12.6)	85.1 ± 0.6 (85)	86.5 ± 0.4 (87)	0.0 ± 0 (0)	0.0 ± 0 (0)

Field values are presented ± 1 SE, and values utilized to program the growth chambers are between brackets. [†]Illuminance in the growth chambers could only be programmed using 'light steps' (LS), corresponding to the number of light bulbs active at each step within the chamber. At 0 LS no bulb is active (0 lx); 1 LS, 1 bulb on (approx. 1800 lx); 2 LS, 2 bulbs on (approx. 3400 lx); 3 LS, 3 bulbs on (approx. 5000 lx); 4 LS, 9 bulbs on (approx. 15000 lx); and 5 LS, 15 bulbs on or all available (approx. 22000 lx).

and interaction terms as fixed factors. Seed mass was included as a covariate given that it can have an effect on germination (Maranon & Grubb 1993; Leishman & Westoby 1994). Pairwise post hoc comparisons were done using chi-square tests (Littell *et al.* 2006; SAS Institute Inc. 2012). The post hoc *P*-values were corrected for multiple comparisons using the false discovery rate procedure (Benjamini & Hochberg 1995). To document the strength of reciprocal effects of the simulated micro-habitat conditions we calculated RII using Equation 1 (Armas *et al.* 2004), wherein D_u corresponded to the condition when the simulated micro-habitat matched the seed source, and D_o corresponded to the condition when seed germination was assessed on the reciprocal simulated micro-habitat. The effect of the simulated micro-habitat on the germination of seeds collected from both micro-habitats was considered positive when $RII > 0$, and negative when $RII < 0$.

RESULTS

Plant density

Micro-habitats and species identity significantly influenced plant density estimates in the field (Fig. 1, Table 2, Appendix S1). *Cyperus hermaphroditus* and *Nassella mucronata* were more abundant in the understorey benefiting from strong facilitative effects by the nurse-plant *Caesalpinia spinosa*. However, *Alonsoa meridionalis* and *Fuertesimilva peruviana* were more abundant in open micro-habitats whilst the abundance of *Plantago limensis* did not significantly differ between micro-habitats.

Seed mass and viability

There were no significant differences in seed mass between understorey and open micro-habitats, but

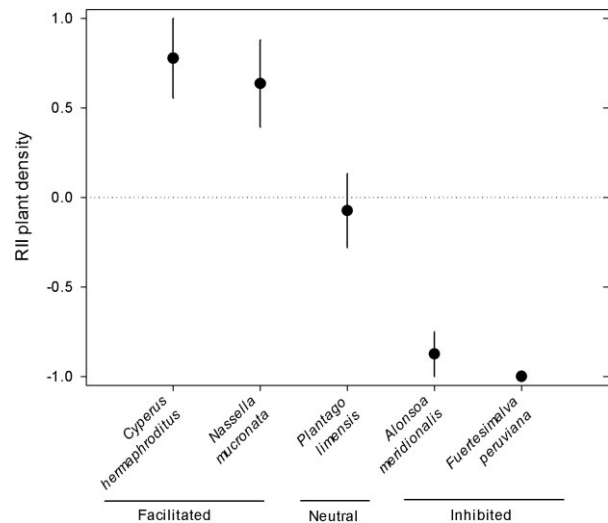


Fig. 1. Relative interaction indices for plant density of five annual plant species growing in open and understorey micro-habitats in Atiquipa, Southern Peru. Means and standard errors are shown.

there were significant differences among species (Table 2, Appendix S2). The percentage of viable seeds was not significantly different between micro-habitats or among species (Table 2, Appendix S2). There were no significant differences in the coefficients of variation for seed mass and seed viability between collection sources (seed mass: $t_8 = 0.7927$, $P = 0.4508$, seed viability: $t_8 = 0.1584$, $P = 0.8781$).

Seed germination

A total of 605 seeds germinated out of the 2000 seeds used in the experiment, that is 30.25% overall germination rate. However, germination rates significantly

Table 2. Summary of generalized linear mixed models of plant density, seed mass and viability for five annual species found in both understorey and open micro-habitats (i.e. seed sources)

Effect	Plant density (d.f. = 440)		Seed mass (d.f. = 190)		Seed viability (d.f. = 30)	
	Chi-square	P-value	Chi-square	P-value	Chi-square	P-value
Species	2223.62	<0.0001	97.25	<0.0001	4.78	0.3103
Source	1514.66	<0.0001	0.05	0.8291	0.00	0.9756
Species*Source	2171.96	<0.0001	0.06	0.9996	0.05	0.9997

Chi-square values are presented along with the corresponding *P*-values. Bolded values indicate statistically significant differences ($P < 0.05$).

Table 3. Summary of generalized linear mixed models of seed germination under controlled conditions (growth chambers)

Effect	d.f.	Germination rate		Days to 50% germination	
		Chi-square	P-value	Chi-square	P-value
Seed mass	1	4.19	0.0405	0.39	0.5349
Micro-habitat	1	14.69	0.0001	1.01	0.3171
Source	1	12.63	0.0004	0.35	0.556
Species	4	116.74	<0.0001	15.36	0.004
Micro-habitat*Source	1	6.28	0.0122	0.02	0.8885
Micro-habitat*Species	4	48.17	<0.0001	2.88	0.5784
Source*Species	4	21.16	0.0003	0.29	0.9903
Micro-habitat*Source*Species	4	19.57	0.0006	0.31	0.9892

Micro-habitat refers to the chamber conditions, while source corresponds to the micro-habitat where seeds were collected. Chi-square values are presented along with corresponding *P*-values. Bolded values indicate statistically significant differences ($P < 0.05$).

differed among species (Table 3), although not between *Cyperus hermaphroditus* and *Fuertesimalva peruviana* (chi-square = 2.4902, $P = 0.1146$). Total germination rates were 42.50% for *Plantago limensis*, 30.50% for *Nasella mucronata*, 18.25% for *Cyperus hermaphroditus* and 6.75% for *Fuertesimalva peruviana*. In addition, germination was significantly higher for seeds collected in open micro-habitats (i.e. significant source effect), and for all seeds regardless of source germinating in simulated open micro-habitat conditions (i.e. significant micro-habitat effect; Fig. 2a, Table 3). However, this result was mainly driven by *Cyperus hermaphroditus*, as effects of seed source and micro-habitat conditions did not differ for the other species (Table 4, Appendix S3). The number of days to 50% germination significantly differed among species (Fig. 2b, Table 3, Appendix S4), but there was no significant difference between sources or simulated micro-habitat conditions (Table 3).

DISCUSSION

We predicted that plant–plant facilitation would have positive effects on the seed biology and germination rates of understorey species. However, there was no evidence in these seed biology analyses or germination

trials of plant facilitation effects on the seed life-stage trait set. Admittedly, only 2 of the 5 species experienced facilitation by nurse-plants at the plant life-stage and this necessarily limits the scope of our findings. Nonetheless, germination was significantly higher for seeds collected in open micro-habitats and most importantly for all species germinating in simulated open micro-habitats including the species that were facilitated by the nurse plant. Furthermore, these general differences were mainly driven by the annual species, *Cyperus hermaphroditus*, the most strongly facilitated species at the plant life-stage we studied, suggesting that plant facilitation does not shape seed biology. In addition, there was no evidence for reduced variability in seed size or viability, and germination was not accelerated for seeds from under nurse-plants. Even with potential maternal effects likely included in the seeds (Luzuriaga *et al.* 2006), no effects of nurse-plants were found on the net outcome of the measured traits. Overall, these results indicate that the effects of the improved micro-habitat conditions generated by nurse-plants had no consistent effects on the seed biology and germinability of understorey species, and do not thus support the hypothesis that relatively more fixed traits from an evolutionary perspective diverge between individuals of a population partitioned between understorey and open microsites. Selection

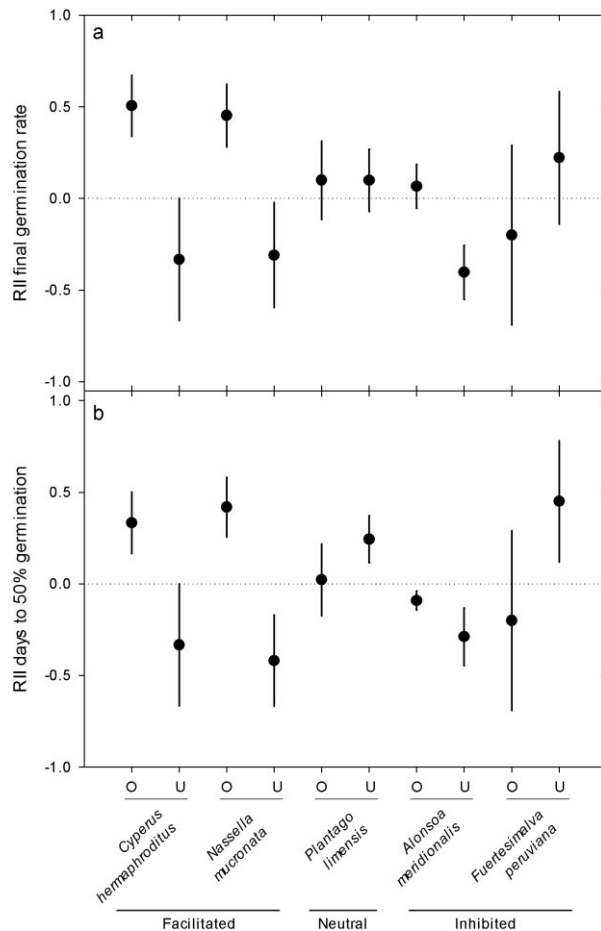


Fig. 2. Relative interaction indices ($RII \pm SE$) for seed germination of five annual plant species collected in open and understorey micro-habitats. Seeds from both sources were germinated under simulated open and understorey conditions in growth chambers. (a) Final germination rates. (b) Numbers of days required to 50% germination.

processes associated with nurse-plant effects on annuals may be greater for vegetative traits.

Our study focused on the effects of nurse-plants, including both strongly facilitated and strongly inhibited plant species. The lack of differences in the traits measured suggests that seed biology traits are very conservative (Moles *et al.* 2005), or that they are controlled by a combination of complex genotype-environment relationships not entirely addressed in our experiment (Finch-Savage & Leubner-Metzger 2006). They also suggest that even while dispersal ability of understorey species is low (Venable *et al.* 2008; Giladi *et al.* 2013), ecotypic differentiation does not occur in this system. Local adaptation is not favoured in stressful conditions and plasticity is a more dominant strategy that allows desert plants to cope with highly unpredictable environmental conditions (Sultan & Spencer 2002; Chesson *et al.* 2004). Conversely, Liancourt and Tielbörger (2011) demonstrated local adaptation for plants from arid environments by subjecting individuals of the same species from Mediterranean conditions to arid field conditions. The contradiction in findings between studies may be related to the length of the gradients under study in that plants may adapt to a variety of conditions via plasticity of traits at local scales (this study), but they develop local adaptations when larger scales/gradients are considered (Liancourt & Tielbörger 2011). Although seed life-stages are critical for annuals, our study presents no evidence for consistent differences in performance between micro-habitats under controlled experimental conditions. Annual species that have more divergent population distributions with less gene flow may also be needed for nurse-plants to generate detectable selection processes, that is for species that are relatively rare in

Table 4. Summary of post hoc chi-square contrasts for seed germination of five annual species

Contrast	<i>Alonsoa meridionalis</i>	<i>Cyperus hemaphroditus</i>	<i>Fuertesimilva peruviana</i>	<i>Nassella mucronata</i>	<i>Plantago limensis</i>
By source					
O-O <i>vs.</i> O-U [†]	0.4592	0.0765	0.7850	0.7850	0.9731
U-U <i>vs.</i> U-O	0.2714	U-O 0.0420	0.2714	0.7850	0.4814
By simulated micro-habitat					
O-O <i>vs.</i> O-U [‡]	0.7850	0.2714	0.7850	0.2714	0.3963
U-U <i>vs.</i> U-O	0.3819	0.0600	0.2714	0.2714	0.7850
Home <i>vs.</i> away					
O-O <i>vs.</i> U-U	0.2714	O-O 0.0420	0.3819	0.8265	0.3819
O-U <i>vs.</i> U-O	0.7423	0.2714	0.9962	0.3819	0.2714

False discovery rate corrected *P*-values are presented. Combinations of seed source (open (O) and understorey (U)) and simulated micro-habitat (open (O) and understorey (U)) were contrasted with their respective reciprocal treatment. Bolded values indicate statistically significant values ($P < 0.05$) along with the corresponding treatment with the higher final germination rate. [†]By source, O-O *versus* O-U: seeds that germinated under open micro-habitat conditions and were collected in open micro-habitat *versus* seeds that germinated understorey micro-habitat conditions and were collected in open micro-habitat. [‡]By micro-habitat, O-O *versus* O-U: seeds that germinated under open micro-habitat conditions and were collected in open micro-habitat *versus* seeds that germinated under open micro-habitat conditions and were collected in understorey micro-habitat.

a system with a net balance toward nurse-plant associations.

We also predicted that the potential selective pressure of increased competition driven by higher plant densities under nurse-plants would lead to adaptive acceleration in germination (Dyer *et al.* 2000; Tielbörger & Kadmon 2000). We did not find evidence to this effect. One explanation is that the strength of apparent competition in the understorey was insufficient to act as a selective process on seed biology characteristics. Generally, no effect of apparent competition on the demographic responses of understorey plants has been demonstrated in other arid ecosystem studies (Tielbörger & Kadmon 2000; Soliveres *et al.* 2011), suggesting that either apparent competition is too infrequent or too weak in these contrasts. Alternatively, the net positive effect of shrubs could also neutralize changes in germination rates associated with avoiding annual plant–plant competition. In this sense, lack of response on seed biology traits could be related to their conservative nature (Moles *et al.* 2005), but could also be due to stabilizing selection generated by counter-directional interactions in a nurse-plant–annual system. A more powerful test of these predictions would be to either sample or generate annual plant density gradients to increase the likelihood that indirect effects are present/persistent enough to impact micro-evolutionary processes.

Germination was favoured for seeds collected in open micro-habitats, and most importantly, for all seeds regardless of species identity germinating under simulated open micro-habitats. This is a compelling finding and an opportunity to reconsider the context-dependency of nurse-plant effects generally assumed in the facilitation literature (Brooker *et al.* 2008; Le Bagousse-Pinguet *et al.* 2013; McIntire & Fajardo 2014). Understorey micro-habitats may not necessarily always represent the most ideal abiotic conditions for annuals to germinate because of low light conditions inducing low photosynthetic rates (Forseth *et al.* 2001; Jensen *et al.* 2011), and lower availability of water and nutrients (Callaway *et al.* 1991; Holzapfel & Mahall 1999). Certainly, the presence of annuals in the understorey is the product of a net positive effect that is the outcome of both positive (e.g. stress amelioration) and negative effects (e.g. reduced resources such as light or water as tested in our experiment) (Holzapfel & Mahall 1999; Callaway 2007); hence the result that seed germination seems to be reduced in understorey conditions might not be entirely unexpected. The seeds of arid annual plant species, or stress tolerant species in general, may also be adapted to higher-stress conditions (Körner 2003) and more rapid or higher germination rates in the context of overarching facilitation is not a signal that they have or can respond. Our results provide a useful insight into the widely assumed view that nurse-plants are clear

examples of positive interactions in stressful environments (Maestre *et al.* 2003; Gomez-Aparicio *et al.* 2004) because evolutionary processes and the trait set in question can be important considerations. We suggest that these novel avenues for research, the seed life-stage and the impact of facilitation on evolutionary processes, be used to structure future facilitation studies in arid ecosystems.

Collectively, we found that nurse-plant positive effects do not necessarily translate into divergent seed characteristics for the understorey plant species growing in both canopies and more open micro-habitats. Apparent competition in nurse-plant canopies may not be sufficiently intense to generate selective processes that overcome facilitation and impact the seed biology and germination of these understorey species. A major implication is that local adaptation and plasticity of beneficiary species are necessary research topics to expand facilitation research. Further ecological studies should also extend the distribution ranges tested and explore density gradients to pinpoint the micro-evolutionary effects of nurse-plants on other species.

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REFERENCES

- Adondakis S. & Venable D. L. (2004) Dormancy and germination in a guild of Sonoran Desert annuals. *Ecology* **85**, 2582–90.
- Armas C., Ordiales R. & Pugnaire F. I. (2004) Measuring plant interactions: a new comparative index. *Ecology* **85**, 2682–6.
- Baskin C. C. & Baskin J. M. (2001) *Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego.
- Benjamini Y. & Hochberg Y. (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J. Roy. Statist. Soc. Ser. B* **57**, 289–300.
- Bertness M. D. & Callaway R. M. (1994) Positive interactions in communities. *Trends Ecol. Evol.* **9**, 191–3.
- Brako L. & Zarucchi J. L. (1993) Catalogue of the flowering plants and gymnosperms of Peru. Monographs in Systematic Botany Vol. 45. Missouri Botanical Garden, St. Louis.
- Breen A. N. & Richards J. H. (2008) Irrigation and fertilization effects on seed number, size, germination and seedling growth: implications for desert shrub establishment. *Oecologia* **157**, 13–19.
- Brooker R. W., Maestre F. T., Callaway R. M. *et al.* (2008) Facilitation in plant communities: the past, the present, and the future. *J. Ecol.* **96**, 18–34.

- Bruno J. F., Stachowicz J. J. & Bertness M. D. (2003) Inclusion of facilitation into ecological theory. *Trends Ecol. Evol.* **18**, 119–25.
- Bullock J. M. & Moy I. L. (2004) Plants as seed traps: inter-specific interference with dispersal. *Acta Oecol.* **25**, 35–41.
- Callaway R. M. (2007) *Positive Interactions and Interdependence in Plant Communities*. Springer, Dordrecht.
- Callaway R. M., Nadkarni N. M. & Mahall B. E. (1991) Facilitation and interference of *Quercus douglasii* on understory productivity in Central California. *Ecology* **72**, 1484–99.
- Chesson P. (2000) Mechanisms of maintenance of species diversity. *Annu. Rev. Ecol. Syst.* **31**, 343–66.
- Chesson P., Gebauer R. L. E., Schwinning S. *et al.* (2004) Resource pulses, species interactions, and diversity maintenance in arid and semi-arid environments. *Oecologia* **141**, 236–53.
- Donohue K. (2009) Completing the cycle: maternal effects as the missing link in plant life histories. *Phil. Trans. R. Soc. B* **364**, 1059–74.
- Dyer A. R., Fenech A. & Rice K. J. (2000) Accelerated seedling emergence in interspecific competitive neighbourhoods. *Ecol. Lett.* **3**, 523–9.
- Ellner S. & Shmida A. (1981) Why are adaptations for long-range seed dispersal rare in desert plants? *Oecologia* **51**, 133–44.
- Facelli J. M., Chesson P. & Barnes N. (2005) Differences in seed biology of annual plants in arid lands: a key ingredient of the storage effect. *Ecology* **86**, 2998–3006.
- Finch-Savage W. E. & Leubner-Metzger G. (2006) Seed dormancy and the control of germination. *New Phytol.* **171**, 501–23.
- Flemion F. & Poole H. (1948) Seed viability tests with 2,3,5 triphenyl tetrazolium chloride. *Contrib. Boyce Thomp. Inst.* **15**, 243–58.
- Flores J. & Jurado E. (2003) Are nurse-protégé interactions more common among plants from arid environments? *J. Vég. Sci.* **14**, 911–16.
- Forseth I. N., Wait D. A. & Casper B. B. (2001) Shading by shrubs in a desert system reduces the physiological and demographic performance of an associated herbaceous perennial. *J. Ecol.* **89**, 670–80.
- Galloway L. F. (2005) Maternal effects provide phenotypic adaptation to local environmental conditions. *New Phytol.* **166**, 93–100.
- Giladi I., Segoli M. & Ungar E. D. (2013) Shrubs and herbaceous seed flow in a semi-arid landscape: dual functioning of shrubs as trap and barrier. *J. Ecol.* **101**, 97–106.
- Goldberg D. E., Turkington R., Olsvig-Whittaker L. *et al.* (2001) Density dependence in an annual plant community: variation among life history stages. *Ecol. Monogr.* **71**, 423–46.
- Gomez-Aparicio L., Zamora R., Gómez J. M. *et al.* (2004) Applying plant facilitation to forest restoration: a meta-analysis of the use of shrubs as nurse plants. *Ecol. Appl.* **14**, 1128–38.
- Holzapfel C. & Mahall B. E. (1999) Bidirectional facilitation and interference between shrubs and annuals in the Mojave Desert. *Ecology* **80**, 1747–61.
- Holzapfel C., Tielbörger K., Parag H. A. *et al.* (2006) Annual plant-shrub interactions along an aridity gradient. *Basic Appl. Ecol.* **7**, 268–79.
- Hufford K. M. & Mazer S. J. (2003) Plant ecotypes: genetic differentiation in the age of ecological restoration. *Trends Ecol. Evol.* **18**, 147–55.
- Jensen A. M., Lof M. & Gardiner E. S. (2011) Effects of above- and below-ground competition from shrubs on photosynthesis, transpiration and growth in *Quercus robur* L. seedlings. *Environ. Exp. Bot.* **71**, 367–75.
- Kéfi S., van Baalen M., Rietkerk M. *et al.* (2008) Evolution of local facilitation in arid ecosystems. *Am. Nat.* **172**, E1–17.
- Körner C. (2003) Limitation and stress: always or never? *J. Vég. Sci.* **14**, 141–3.
- Le Bagousse-Pinguet Y., Forey E., Touzard B. *et al.* (2013) Disentangling the effects of water and nutrients for studying the outcome of plant interactions in sand dune ecosystems. *J. Vég. Sci.* **24**, 375–83.
- Leishman M. R. & Westoby M. (1994) The role of seed size in seedling establishment in dry soil conditions – experimental evidence from semi-arid species. *J. Ecol.* **82**, 249–58.
- Liancourt P., Callaway R. M. & Michalet R. (2005) Stress tolerance and competitive response ability determine the outcome of biotic interactions. *Ecology* **86**, 1611–18.
- Liancourt P. & Tielbörger K. (2011) Ecotypic differentiation determines the outcome of positive interactions in a dryland annual plant species. *Perspect. Plant Ecol. Evol. Syst.* **13**, 259–64.
- Littell R. C., Milliken G. A., Stroup W. W. *et al.* (2006) *SAS for Mixed Models*. SAS Institute Inc., Cary.
- Luzuriaga A. L., Escudero A. & Pérez-García F. (2006) Environmental maternal effects on seed morphology and germination in *Sinapis arvensis* (Cruciferae). *Weed Res.* **46**, 163–74.
- McIntire E. J. B. & Fajardo A. (2014) Facilitation as a ubiquitous driver of biodiversity. *New Phytol.* **201**, 403–16.
- Maestre F. T., Bautista S. & Cortina J. (2003) Positive, negative and net effects in grass-shrub interactions in Mediterranean semiarid grasslands. *Ecology* **84**, 3186–97.
- Maestre F. T., Callaway R. M., Valladares F. *et al.* (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *J. Ecol.* **97**, 199–205.
- Maranon T. & Grubb P. J. (1993) Physiological basis and ecological significance of the seed size and relative growth rate relationship in Mediterranean annuals. *Funct. Ecol.* **7**, 591–9.
- Maron J. L., Vila M., Bommarco R. *et al.* (2004) Rapid evolution of an invasive plant. *Ecol. Monogr.* **74**, 261–80.
- Moles A. T., Ackerly D. D., Webb C. O. *et al.* (2005) Factors that shape seed mass evolution. *Proc. Natl Acad. Sci. USA* **102**, 10540–4.
- Noy-Meir I. (1973) Desert ecosystems: environment and producers. *Annu. Rev. Ecol. Syst.* **4**, 25–51.
- Pake C. E. & Venable D. L. (1996) Seed banks in desert annuals: implications for persistence and coexistence in variable environments. *Ecology* **77**, 1427–35.
- Pugnaire F. I., Haase P., Puigdefabregas J. *et al.* (1996) Facilitation and succession under the canopy of a leguminous shrub: *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos* **76**, 455–64.
- Pugnaire F. I. & Lazaro R. (2000) Seed bank and understory species composition in a semi-arid environment: the effect of shrub age and rainfall. *Ann. Bot.* **86**, 807–13.
- Roach D. A. & Wulff R. D. (1987) Maternal effects in plants. *Annu. Rev. Ecol. Syst.* **18**, 209–35.
- SAS Institute Inc (2012) *JMP 10 Modeling and Multivariate Methods*. SAS Institute Inc., Cary.
- Seifan M., Tielbörger K. & Kadmon R. (2010) Direct and indirect interactions among plants explain counterintuitive positive drought effects on an eastern Mediterranean shrub species. *Oikos* **119**, 1601–9.

- Soliveres S., Eldridge D. J., Maestre F. T. *et al.* (2011) Microhabitat amelioration and reduced competition among understory plants as drivers of facilitation across environmental gradients: towards a unifying framework. *Perspect. Plant Ecol. Evol. Syst.* **13**, 247–58.
- Sotomayor D. A. & Jimenez P. (2008) Condiciones meteorológicas y dinámica vegetal del ecosistema costero Lomas de Atiquipa (Caraveli-Arequipa) en el sur del Perú. *Ecología Aplicada* **7**, 1–8.
- Sprague T. A. (1931) The botanical name of ‘Tara’. *Kew Bull. Misc. Inform.* **1931**, 91–6.
- Sultan S. E. (1996) Plasticity for offspring traits in *Polygonum persicaria*. *Ecology* **77**, 1791–807.
- Sultan S. E. & Spencer H. G. (2002) Metapopulation structure favors plasticity over local adaptation. *Am. Nat.* **160**, 271–83.
- Thorpe A. S., Aschehoug E. T., Atwater D. Z. *et al.* (2011) Interactions among plants and evolution. *J. Ecol.* **99**, 729–40.
- Tielbörger K. & Kadmon R. (2000) Indirect effects in a desert plant community: is competition among annuals more intense under shrub canopies? *Plant Ecol.* **150**, 53–63.
- Valencia-Díaz S. & Montaña C. (2005) Temporal variability in the maternal environment and its effect on seed size and seed quality in *Flourensia cernua* DC. (Asteraceae). *J. Arid Environ.* **63**, 686–95.
- Venable D. L. (2007) Bet hedging in a guild of desert annuals. *Ecology* **88**, 1086–90.
- Venable D. L., Flores-Martinez A., Muller-Landau H. C. *et al.* (2008) Seed dispersal of desert annuals. *Ecology* **89**, 2218–27.
- Venable D. L. & Lawlor L. (1980) Delayed germination and dispersal in desert annuals: escape in space and time. *Oecologia* **46**, 272–82.
- Ward D. (2009) *The Biology of Deserts*. Oxford University Press, New York.
- Whitford W. G. (2002) *Ecology of Desert Systems*. Academic Press, San Diego.
- Wilson M. F. (1993) Dispersal mode, seed shadows and colonization patterns. *Végétatio* **108**, 261–80.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Density of five annual species in open and understorey micro-habitats.

Appendix S2. Seed attributes of the five studied species.

Appendix S3. Germination rates for seeds of five annual species collected in different micro-habitats.

Appendix S4. Time to 50% germination for seeds of five annual species collected in different micro-habitats.