Functional Ecology

British Ecological Society

Functional Ecology 2016 doi: 10.1111/1365-2435.12660

Same nurse but different time: temporal divergence in the facilitation of plant lineages with contrasted functional syndromes

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Summary

1. Plant facilitation assembles functionally contrasted communities in dry lands. Nurse plants are often early colonizers with xerophytic traits that were mostly selected during the dry Quaternary (Quaternary syndrome), while beneficiary plants tend to be late-successional species with mesophytic traits that evolved mainly during the more humid Tertiary (Tertiary syndrome). Integrating plant facilitation within the community assembly theory requires a better understanding of the ontogenetic development of the nursing abilities that benefit functionally contrasted species.

2. We assessed whether the same nurse plant facilitates species with Quaternary and Tertiary syndromes in an ecosystem under severe abiotic stress conditions imposed by aridity and gypsum soil toxicity. We hypothesized that both functional types find suitable microsites for seedling establishment underneath the same nurse but their optimal regeneration niches are temporally segregated along the ontogenetic development of the nurse.

3. We carried out a sowing experiment along a 40-year ontogenetic gradient of the nurse shrub *Ononis tridentata*. Seeds from five Tertiary and five Quaternary species were sown and seedling emergence monitored.

4. While the nurse age did not affect the seedling emergence of Quaternary species, it significantly increased that of Tertiary species. These results were corroborated for elder ontogenetic stages in non-manipulated plants in the field. Juveniles of Quaternary species were able to grow beneath nurse plants along their whole ontogenetic gradient excepting beneath *Ononis* seedlings, while Tertiary species were only facilitated by mature nurses.

5. *Synthesis*. Our results show that plant nursing abilities evolve ontogenetically in a different way for beneficiary Quaternary and Tertiary plant lineages. The finding of a plant species that plays a role as key assembler of early- and late-successional species in plant dynamics broadens the scope of facilitation in the community assembly theory.

Key-words: abiotic stress, community assembly, island of fertility, ontogenetic gradient, patchy ecosystem, plant–plant interactions, seedling emergence, soil fertility

Introduction

Facilitation is a major force shaping plant communities in dry lands that tends to assemble functionally contrasted species (Brooker *et al.* 2008; Butterfield & Briggs 2011; Soliveres, Smit & Maestre 2015). Nurse plants often bear drought-tolerant traits that allow them recruiting in barren sites, such as dry fruits, small seeds, trichome lining, low specific leaf area, summer deciduous leaves, low secondary rooting or dominance of tap roots. These nurse plants generate the microsites that fulfil the regeneration niche requirements of the facilitated species that are less stress-tolerant (Valiente-Banuet & Verdú 2013; McIntire & Fajardo 2014). Indeed, beneficiary species generally have mesophytic traits that restrict their opportunities for successfully establishing in open areas, such as bird-dispersed fleshy fruits, bigger seeds or higher specific leaf area (Herrera 1992; Díaz & Cabildo 1997; Joffre, Rambal &

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Damesin 1999; Cornelissen *et al.* 2003; Butterfield & Briggs 2011). The contrasted functional syndromes between the nurse and its beneficiary species have been attributed to the differential environmental conditions during lineage evolution. While the xerophytic traits of early colonizers were selected during the climate aridization process that occurred in the Quaternary, the mesophytic traits of facilitated species evolved during the more humid Tertiary (Valiente-Banuet *et al.* 2006). For this reason, in dry lands a match is assumed between the functional syndrome in terms of regeneration niche (xerophytic vs. mesophytic traits) and the lineage age (Quaternary vs. Tertiary lineages) (Herrera 1992). For instance, in Mediterranean-type ecosystems 81% of Quaternary lineages are early-successional species whereas 98% of Tertiary lineages are late-successional species (Valiente-Banuet *et al.* 2006). Thus, facilitation has played a central role in the preservation of ancient Tertiary plant lineages that have benefited from modern Quaternary lineages under stressful conditions (Valiente-Banuet *et al.* 2006). In the literature, there is also some, though scarce, evidence of facilitation between species with a Quaternary syndrome, for example *Lotus salsuginosus* Greene., *Cercocarpus betuloides* Nutt. and *Purshia glandulosa* Curran (Californian chaparral), *Globularia alypum* L. and *Teucrium capitatum* L. (Mediterranean Basin), *Anthospermum spathulatum* L. (South African Fynbos) (Valiente-Banuet *et al.* 2006). Plant species able to facilitate both Quaternary and Tertiary species have not been explicitly considered in the literature.

The net outcome of interspecific plant–plant interactions depends on multiple abiotic and biotic factors. While the stress gradient hypothesis predicts a direct relationship between the magnitude of abiotic stress and the importance of facilitation (Bertness & Callaway 1994), there is evidence indicating that severe stress conditions may turn facilitative interactions into competition (Tielbörger & Kadmon 2000; Maestre & Cortina 2004; Michalet *et al.* 2006). The type and local distribution of abiotic stress factors and the functional traits of the species also add variability to the net response of interspecific relationships (Tielbörger & Kadmon 2000; Kawai & Tokeshi 2007; Maestre *et al.* 2009; Butterfield & Callaway 2013; Soliveres, Smit & Maestre 2015). In the last decade, several authors have highlighted the necessity of assessing the whole life cycle of interacting species to better estimate the net effects of an interaction (e.g. Miriti 2006; Valiente-Banuet & Verdú 2008; Soliveres *et al.* 2010; Le Roux, Shaw & Chown 2013). Most surveys on facilitation have tested the effect of adult nurse plants on different ontogenetic stages of its beneficiaries. Interestingly, the phylogenetic distance to the nurse is a main factor determining seedling establishment of facilitated plants owing to the reduced niche overlap between distantly related species (Castillo, Verdú & Valiente-Banuet 2010). The few cases in which the nurse size was taken into account as a proxy of its ontogenetic development reported shifts in the facilitated seedling establishment, adult fitness, species richness and/or composition along with the reduction of abiotic stress (e.g. Kellman & Kading 1992; Pugnaire *et al.* 1996; Pugnaire & Lazaro 2000; Tewksbury & Lloyd 2001). These observations suggest that plants with contrasted functional syndromes may be differentially facilitated throughout the ontogenetic evolution of the nurse.

In Mediterranean semi-arid gypsum soils, we have observed that adults of the early colonizer gypsophyte *Ononis tridentata* L. act as nurse plants that facilitate the establishment of *c.* 40 less gypsum-tolerant species, thus developing multispecific plant patches (Navarro-Cano *et al.* 2014). We have also recorded how this interaction ameliorates the double abiotic stress (water limitation and soil toxicity) and forms fertility islands from the establishment of *O. tridentata* seedlings on bare soils up to 40 year-old patches (Navarro-Cano *et al.* 2015). This survey suggested a significant contribution of the facilitated community to the improvement of soil productivity triggered by the nurse species. Finally, in the field we observed clues of co-occurrence of Quaternary and Tertiary plant lineages underneath adults of *O. tridentata*. Based on these observations, we hypothesized that despite their coexistence below the same nurse plant, the optimal regeneration niche of Quaternary and Tertiary beneficiary species might be temporally segregated along the nurse life span. This hypothesis requires that (i) the abiotic conditions below the nurse plant evolve with the nurse age and (ii) these changes match with the differential functional syndromes (i.e. xerophytic vs. mesophytic) of beneficiary species. Thus, we expected early colonizers to establish better below young nurses and late colonizers below old nurses. A theoretical model of our hypothesis linking soil development and nursing ability is shown in Fig. 1. To test our hypothesis we aimed to (i) assess the shifts in seedling emergence of a set of species with Quaternary and Tertiary syndromes sown along a gradient of *O. tridentata* ontogenetic development (i.e. from young to old individuals) and (ii) check whether the facilitation pattern observed at the establishment stage under manipulative conditions is mirrored in the subsequent ontogenetic stages under natural conditions. Confirmation of contrasted strength of facilitation of an early colonizer species on both early- and late-successional species along the nurse age would motivate a revision of the role of facilitative interactions on community assembly rules when several abiotic stresses concur (Bruno, Stachowicz & Bertness 2003; Soliveres, Smit & Maestre 2015).

Materials and methods

STUDY SYSTEM

We selected a patchy ecosystem in a hilly landscape in Serra de Crevillent (Alacant, SE Spain; 38°16′15′′ N, 0°50′16′′W; average 350 m a.s.l., 30% slope). Climate is semi-arid Mediterranean (240 mm mean annual rainfall, 20 °C mean annual temperature).

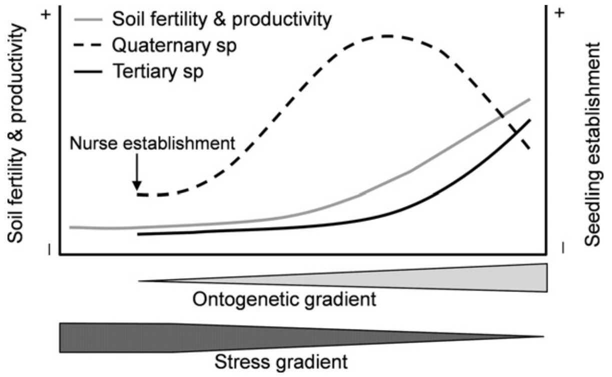

Fig. 1. Theoretical model suggested to integrate environmental and functional traits into the community assembly process in facilitation-driven ecosystems. The nurse establishment on barren soil and its ontogenetic development trigger the physical (temperature, radiation, water availability), chemical (soil fertility parameters) and microbial (soil microbial productivity) amelioration of microsites beneath the nurse, which gradually reverse the stress gradient for plant recruitment. This allows the establishment of Quaternary lineages or species with a Quaternary syndrome (xerophytic traits). Quaternary species join the nurse–soil feedbacks, thus accelerating microsite changes. Finally, the patchy system reaches the conditions for the establishment of Tertiary lineages or species with a Tertiary syndrome (mesophytic traits), thus enabling the assembly of both early- and late-successional species below the same nurse plant species.

Soils are Typic Xerorthents developed on gypsum outcrops, which are young soils with properties hardly differentiated from their parent material (Goberna *et al.* 2007). Vegetation is dominated by one of the most frequent gypsophyte legume shrubs in the Iberian Peninsula *Ononis tridentata* (Mota, Sánchez-Gómez & Guirado 2011). *Ononis* promotes a patch-gap mosaic with an overall 25% plant cover in the area (Navarro-Cano *et al.* 2015). The gaps are covered by sealing crusts with some gypsophyte chamaephytes. *Ononis* seedlings establish on unfertile gaps and increase soil fertility along their ontogenetic development (Navarro-Cano *et al.* 2015). The patches promoted by *Ononis* mainly attract non-gypsophyte dwarf shrubs, perennial grasses and herbs. A checklist of patch- and gap-associated species is available in Navarro-Cano *et al.* (2014).

We selected 50 patches representing a gradient in the age of the *Ononis* nurse. The first five patches in the gradient were barren gaps, and the rest of patches were selected to cover the whole range of ages of the nurse. Barren gaps patches were randomly selected areas of uncolonized bare soil within the two-hectare study area. The 45 *Ononis* patches had been previously characterized by Navarro-Cano *et al.* (2015) and were selected based on the mean diameter of the *Ononis* canopy, which ranged from 26 to 236 cm. Size–growth ring relationships allowed age assessment of *Ononis* individuals, which ranged from 5 to 38 years old. Thus, the 50 plots (45 patches + 5 gaps) were interpreted as an *Ononis* ontogenetic gradient, the five gaps representing the antecedent situation to the establishment of *Ononis*. The gradual rise of different soil fertility and microbial productivity parameters along the same ontogenetic gradient has been reported by Navarro-Cano *et al.* (2015). Principal component analysis (PCA) was used to reduce several parameters related to soil fertility (total organic carbon, total nitrogen, phosphorus, potassium and gravimetric humidity) to a single variable. The first PC was interpreted as a soil fertility gradient, whose increase as a function of *Ononis* age is shown in Fig. 2.

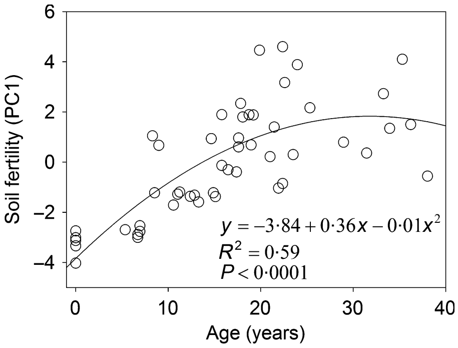

Fig. 2. Soil fertility as a function of *Ononis* age along the studied ontogenetic gradient. Soil fertility was defined as the first principal component of the principal component analysis used to reduce TOC, N, P, K and gravimetric humidity to a single variable. A quadratic fit, explained variance and significance of the *F*-test are shown.

SEED SOWING EXPERIMENT

We performed a seed sowing experiment along the *Ononis* ontogenetic gradient using 10 plant species, five with a Quaternary syndrome and five with a Tertiary syndrome (Table 1). Seeds from all the species were collected in SE Spain from populations under Mediterranean semi-arid climate (240–330 mm mean annual rainfall, 17–20 °C mean annual temperature). The 10 species were naturally present in or near the study area, but only *Rhamnus lycioides* and *Helychrysum stoechas* were present in the studied gypsum outcrop, in a way that confounding seedling emergence from sown and natural seeds was minimized. To guarantee a regular distribution of each species' sowing across the gradient, we grouped the 50 plots into 10 categories (five plots each) of increasing *Ononis* mean diameter (from 0 to 236 cm). One Tertiary and one Quaternary species were sown in random pairs along two 30-cm-long parallel transects in each plot. Twenty-five seeds per species and plot (250 seeds per species along the gradient) were sown regularly spaced along each transect in the uppermost centimetre and totally covered by soil to avoid surface run-off in case of rainfall, heavy wind or other disturbance agents. Seed sowing was carried out on 15 December 2012, which roughly matches with the start of the emergence season in the study area. As the period 1 December 2013–10 February 2013 was drier than the historical average of the last decade in the area (Fig. S1, Supporting information), a supplementary watering was provided on February 2013. Watering was applied on February 11th, 12th and 14th by manual spraying at 1·5 m height above the soil surface on a 0·5 × 0·5 m area with the two parallel sowing transects in the centre of the area. In total, 3·7 L plot^{-1} day^{-1} was poured in two series with 1-h interval. Overall 11 L plot^{-1} was supplied to mimic the average mean monthly rainfall in the area.

Seedling emergence was monitored weekly up to the end of the emergence season on May 2013. During the experiment, we characterize the microenvironmental conditions along the gradient by measuring soil surface temperature, radiation and soil gravimetric humidity at midday on 22 January 2013, 8 March 2013 and 15 April 2013. Temperature was measured with a thermometer CRISON 638Pt (Alella, Spain). Radiation was measured with a sensor type SKP 210 (Skye Instruments Ltd., Wales, UK). Gravimetric humidity content was calculated as the weight loss after 65 °C oven-drying soil samples. Figure 3 shows the mean soil surface temperature, radiation and soil gravimetric humidity at midday during the mentioned period.

The effects of the regeneration niche (Quaternary vs. Tertiary) and *Ononis* age on seedling emergence were analysed by general

Table 1. Differential traits of study species with regard to their regeneration niche and some dispersal traits

Species	Syndrome*	Fruit type*	Seed size† (mm)	Require nurse*
Nurse sp.				
Ononis tridentata L.	Quaternary	Nonfleshy	1·8–2·5	No
Sown sp.				
Anthyllis cytisoides L.	Quaternary	Nonfleshy	1·2 × 2	No
Helianthemum almeriense Pau	Quaternary	Nonfleshy	1·2–1·5	No‡
Helichrysum stoechas (L.) Moench	Quaternary	Nonfleshy	0·3 × 0·5	?
Lavandula dentata L.	Quaternary	Nonfleshy	1·5 × 0·8	?
Thymus hyemalis Lange	Quaternary	Nonfleshy	0·6–1·2	?
Olea europaea L.	Tertiary	Fleshy	10·5 × 5·5	Yes
Pistacia lentiscus L.	Tertiary	Fleshy	4·5 × 4×2·5	Yes
Quercus coccifera L.	Tertiary	Fleshy	23 × 14	Yes
Rhamnus alaternus L.	Tertiary	Fleshy	4·5 × 3	Yes
Rhamnus lycioides L.	Tertiary	Fleshy	4 × 2·5	Yes

*Valiente-Banuet *et al.* (2006).
†From seeds used in this experiment.
‡Nurse requirement has been checked in other species belonging to the same genus.

ized linear mixed models (GLMMs). Seedling emergence was coded as a binary variable representing the proportion of seeds that succeeded and failed to emerge. We used the regeneration niche as a fixed factor, *Ononis* age as a fixed covariate and both plot and species (nested within plot) as random factors. We used a quasibinomial error distribution to account for data overdispersion (Crawley 2005). GLMMs were performed with the glmmPQL function in the package MASS of R v3.1.2 (R Core Team 2014). To explore the variation in seedling emergence due to the functional and phylogenetic variability within each group (i.e. Quaternary and Tertiary species, separately), similar GLMMs were used but including both seed size (a key trait related to seedling emergence) and the phylogenetic distance of each species to the nurse as fixed covariates. Seed size was measured in 30 seeds per species. The phylogenetic distance of each species to *O. tridentata* was calculated with the *cophenetic* function in R based on the phylogenetic tree reconstructed by Navarro-Cano *et al.* (2014).

NATURAL PATTERN OF FACILITATION

We checked whether the facilitation pattern observed at the establishment stage under manipulative conditions was mirrored in the subsequent ontogenetic stages in the study site. We recorded the presence/absence of juveniles of species with Quaternary and Tertiary syndromes that naturally grow in the study area underneath 100 *Ononis* individuals comprising an age gradient from seedlings to mature plants. We focused on beneficiary juveniles to avoid confusion on nurse assignment when perennial species grow in the same patch as *Ononis*. The probability of the presence of Quaternary and Tertiary species naturally facilitated by *Ononis* along its ontogenetic gradient was fitted by logistic regression models using the package STATS of R, and the logistic regression curves were depicted with the package GRAPHICS in R.

Results

SEED SOWING EXPERIMENT

Seedling emergence was observed in eight of 10 sown species (Fig. S2). The Tertiary species *Quercus coccifera* and *Olea europaea* yielded no emergence and thus were not further considered. Overall, Quaternary species had higher mean emergence ± SE (11·4 ± 2·1%) than Tertiary species (2·1 ± 0·8%). The regeneration niche (Quaternary vs. Tertiary species) significantly affected the probability of emergence (Table 2). While *Ononis* age had no effect on seedling emergence, the interaction regeneration niche × *Ononis* age had a significant effect on seedling emergence. When Quaternary and Tertiary species were analysed separately, *Ononis* age did not have an effect on the seedling emergence of Quaternary species (glmmPQL: $t = 0{\cdot}244$, $P = 0{\cdot}8$, value = 0·005 ± 0·022, Fig. 4), whereas seedling emergence of Tertiary species significantly increased with *Ononis* age (glmmPQL: $t = 3{\cdot}403$, $P = 0{\cdot}002$, value = 0·043 ± 0·013, Fig. 4). Once the seed size and phylogenetic distance were added to the models, the results showed that seed size did not affect seedling emergence of Tertiary species, whereas it positively affected the emergence of Quaternary species (Table 3). The phylogenetic distance of the sown species to the nurse plant had a significant positive effect on seedling emergence of Quaternary species and only marginal on the Tertiary species (Table 3). Individually, Quaternary species showed very different emergence patterns along the gradient whereas Tertiary species were more constant refusing emergence under young *Ononis* plants (Fig. S2).

NATURAL PATTERN OF FACILITATION

Wild juvenile Quaternary plants beneath *Ononis* patches were present along the whole ontogenetic gradient excepting the smaller *Ononis* plants (<24 cm height, 31 cm diameter and 6–7 years old), while Tertiary species were absent in *Ononis* plants up to 66 cm height, 105 cm diameter and 17–18 years old (Fig. 5). *Brachypodium retusum* (Pers.) P. Beauv., *Stipa parviflora* Desf., *Fagonia cretica* L., *Teucrium libanitis* Schreb. and *Thymus moroderi* Pau ex Martinez

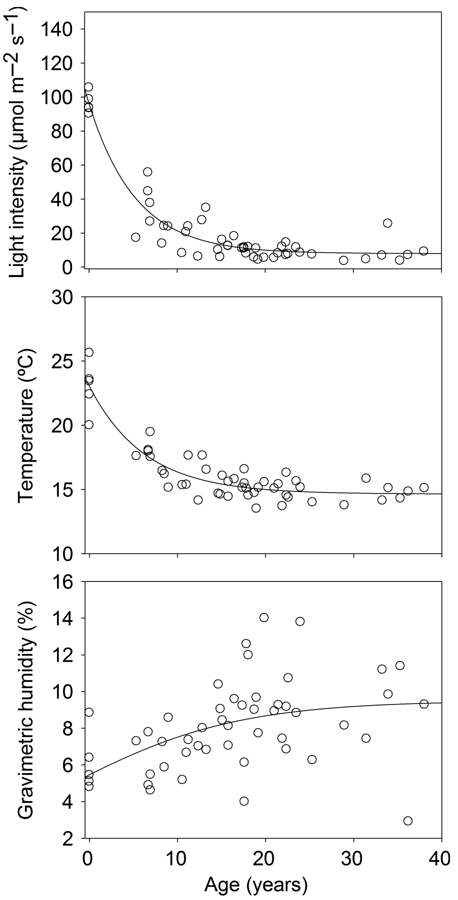

Fig. 3. Microsite characterization of *Ononis* patches along the studied ontogenetic gradient during the period of seedling emergence. Values are means of the measurements carried out in 22 January 2013, 8 March 2013 and 15 April 2013. Regression fits for mean light intensity (exponential decay curve; R^2 = 0·92; P < 0·0001), mean soil surface temperature (exponential decay curve; R^2 = 0·85; P < 0·0001) and mean gravimetric humidity (sigmoidal curve; R^2 = 0·20; P < 0·0047) are shown. Analytical methods are given in the main text.

Table 2. Seedling emergence as a function of regeneration niche (Quaternary/Tertiary) and *Ononis* age. Plot and species (nested within plot) were considered as random factors in the glmmPQL model

	Value	SE	d.f.	*t*-value	*P*
Intercept	−2·996	0·402	48	−7·455	<0·0001
Regeneration niche	−3·573	0·490	28	−7·298	<0·0001
Age	0·016	0·021	48	0·764	0·4486
Regeneration niche × Age	0·088	0·026	28	3·444	0·0018

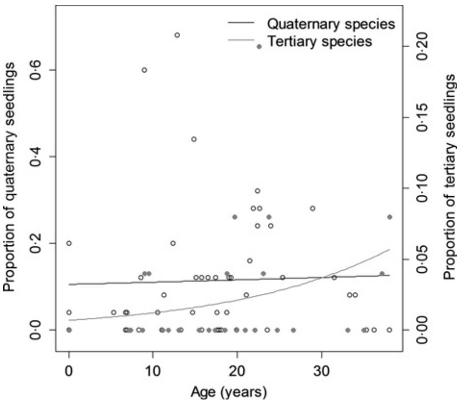

Fig. 4. Proportion of emerged seedlings of five Quaternary species and three Tertiary species sown along an *Ononis* ontogenetic gradient. A logistic fit is depicted in both cases although the positive effect of *Ononis* age on the seedling emergence was only significant for Tertiary species. The results of the generalized linear mixed models are shown in the text.

Table 3. Seedling emergence of Quaternary and Tertiary species as a function of *Ononis* age, seed size and phylogenetic distance to the nurse. Plot and species (nested within plot) were considered as random factors in the glmmPQL models

	Value	SE	d.f.	*t*-value	*P*
Quaternary species					
Intercept	−7·635	1·736	46	−4·398	0·0001
Age	0·009	0·021	46	0·423	0·6742
Seed size	2·242	0·744	46	3·012	0·0042
Phylogenetic distance	0·014	0·004	46	3·520	0·0010
Tertiary species					
Intercept	−22·650	12·967	26	−1·747	0·0925
Age	0·112	0·045	26	2·483	0·0198
Seed size	−2·830	2·160	26	−1·310	0·2016
Phylogenetic distance	0·126	0·070	26	1·791	0·0850

were the five most abundant species among 24 facilitated species with Quaternary syndrome, whereas the facilitated species with Tertiary syndrome were *Rhamnus lycioides* and *Asparagus horridus* L. We estimated that the probability of presence of Quaternary species increases during the first 10 years of *Ononis* and reaches saturation in nurses older than 12–15 years old. That is to say, the probability of presence of Tertiary species is temporarily delayed compared to that of Quaternary species, reaching a probability *c*. 60% for *Ononis* plants with more than 35 years old (Fig. 6).

Discussion

Our sowing experiment provides evidence that the Quaternary species *Ononis tridentata* has the ability to promote the establishment of both Quaternary and Tertiary species. The facilitated Quaternary species showed wider regeneration niches than Tertiary species along the *Ononis* ontogenetic gradient, whereas the Tertiary species were more dependent on the mesic conditions created by older *Ononis* patches. These results go beyond the seedling stage as they were confirmed for subsequent ontogenetic stages by an observational survey on unmanipulated *Ononis* patches in the same study area.

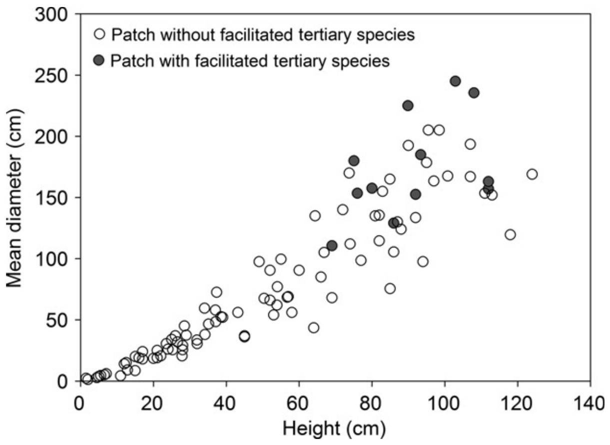

Fig. 5. Presence of Tertiary species naturally facilitated by *Ononis* as a function of *Ononis* height and mean diameter ($n = 100$). The facilitated species with a Tertiary syndrome were *Rhamnus lycioides* and *Asparagus horridus*. All *Ononis* plants excepting those with height <24 cm and diameter <31 cm facilitated Quaternary species.

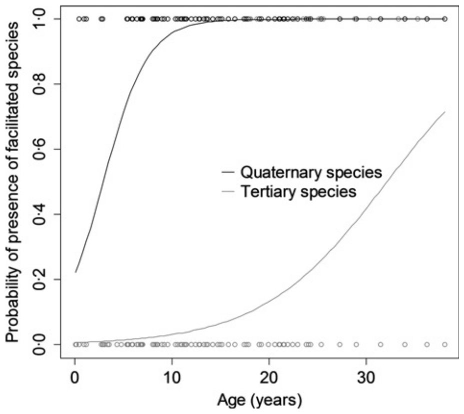

Fig. 6. Presence of naturally facilitated species with Quaternary and Tertiary syndromes as a function of *Ononis* age ($n = 100$). The predicted curves of probability of presence are also shown (Quaternary species, glm: $t = 3·33$, $P < 0·001$; Tertiary species, glm: $t = 3·595$, $P < 0·001$).

Most examples of plant–plant facilitation in Mediterranean environments have species with a Tertiary syndrome as beneficiary plants whereas facilitation between Quaternary species is infrequent in the literature. Valiente-Banuet *et al.* (2006) reported that only 11 species of 107 with a Quaternary syndrome recorded across Mediterranean climate areas of the world require a nurse plant for establishment. Others have also reported a positive co-occurrence between Quaternary species both in the wild and in experiments (e.g. Shmida & Whittaker 1981; Lloret, Peñuelas & Estiarte 2005). Our results join to these cases of facilitation between Quaternary species. In our semi-arid gypsum ecosystem, several non-resource stress factors such as salinity, soil toxicity and high temperatures during summer concur with the existence of a resource-related abiotic stress derived from limiting water and nutrients. Light intensity has a double role, acting as a resource for plant activity, but above certain limits exerting negative genetic, physiological and tissue effects that can be particularly harmful when combined with drought or high temperatures (Suzuki *et al.* 2014). *Ononis tridentata* shows functional traits typical of xerophytic species that function as nurse plants. This species is a pioneer shrub with succulent and summer deciduous leaves (Navarro-Cano *et al.* 2015) with a dominant tap root and low root density (De Baets *et al.* 2007). Similarly, all Quaternary beneficiary species tested here have xerophytic traits, such as a small seed size. Still, differences in seed size among facilitated Quaternary species determined seedling establishment, species with larger seeds being more successful likely due to their higher amount of resources (Butterfield & Briggs 2011). In the adult stage, these species are functionally dissimilar to the nurse mainly because of their smaller aboveground size and shallower root structure (Guerrero-Campo *et al.* 2006; Navarro-Cano *et al.* 2015). Some Tertiary species studied here show some xerophytic traits (e.g. low leaf surface area, high leaf mass per area), but differ from the Quaternary species in their regeneration niche (Navarro-Cano *et al.* 2014) and in physiological traits associated with the stomatal regulation intensity and water use strategy that demand milder stress conditions (Moreno-Gutiérrez *et al.* 2012). These functional complementarities between the nurse and its Quaternary and Tertiary beneficiaries, together with the observation that seedling emergence was promoted with the phylogenetic distance to the nurse, agree with the idea that the strength of facilitation increases as niche overlap decreases (Carrick 2003; Valiente-Banuet & Verdú 2013).

The microenvironmental change of stress factors such as temperature, gravimetric humidity and soil fertility with the nurse age allows the use of our ontogenetic gradient as a stress gradient. The rise of both seedling emergence and presence of juveniles for Tertiary species along the nurse ontogenetic gradient fits the hypothesized model (Fig. 1). This was not the case of Quaternary species, whose emergence would theoretically decrease in the older part of the *Ononis* ontogenetic gradient. This disagreement can be due to a narrower stress gradient encompassed by the studied ontogenetic gradient compared to the theoretical model (Callaway 2007). In this way, the extremely stressful starting conditions due to water limitation, thermal stress, soil toxicity by excessive sulphate ions and nutrient limitation by low organic matter and ion imbalance promoted by high sulphur and calcium concentration (Merlo, Mota & Sánchez-Gómez 2011; Pueyo *et al.* 2011) were significantly relaxed by the nurse species. Nevertheless, this shift likely did not allow reaching sufficiently mesic conditions to turn facilitation into competition between the nurse and the beneficiary Quaternary species or even between Tertiary and Quaternary species, as suggested by Pugnaire *et al.* (1996) in a *Retama sphaerocarpa* (L.) Boiss. age gradient. Both in the sowing experiment and the observational survey, we avoided sampling *Ononis* patches with adult Tertiary species in order to prevent mistargeting of the nurse species. Sampling those patches would have possibly meant a wider stress gradient, thus allowing the detection of changes towards negative effects of interaction in large and old *Ononis* patches where the Tertiary beneficiary species not only contribute to a shadier microenvironment but also to a dominant subcanopy layer occupied by competitive species. This circumstance has been observed in our study system (Navarro-Cano *et al.* 2014) where adult patches are mainly covered beneath the nurse by the rhizomatous perennial grass *Brachypodium retusum* (Navarro-Cano *et al.* 2014). This species has a rapid clonal growth that yields dense and extensive grasslands (Caturla *et al.* 2000; De Luis *et al.* 2004). The same negative interaction has been tested in other semi-arid environments where Quaternary species as *Cistus heterophyllus* Desf. lose regeneration microsites in favour of *B. retusum* when this grass is dominant (Navarro-Cano 2008).

Adding a temporal perspective to the nursing ability of a species is not frequent in the literature on facilitation, which historically has moved from testing interactions between adults or the effect of adult nurses on seedling establishment to assess the net effects of interactions as the beneficiary species evolve ontogenetically (Callaway 2007; Brooker *et al.* 2008). Among the reported effects of nurse age or size on facilitation, Kellman & Kading (1992) observed facilitation of two pine species beneath the canopy of *Quercus rubra* L. in a sand dune succession. This pattern was only confirmed in oaks older than 35 years. Pugnaire *et al.* (1996) and Pugnaire & Lazaro (2000) reported increased plant richness and seed bank density beneath an age gradient of *Retama sphaerocarpa* in a Mediterranean semi-arid area. They observed a spatial segregation of beneficiary plants beneath the nurse canopy, by which drought-resistant species were relegated from the centre of the understorey by more mesic species over the age gradient. Tewksbury & Lloyd (2001) reported a positive effect of the age of the desert tree *Olneya tesota* Gray on the fitness of perennial beneficiary species below their canopy compared to gaps both in xeric and mesic sites. At the community level, Siles *et al.* (2008) reported an example of recovery of plant secondary successional trajectory assisted by facilitative interactions in a mesic Mediterranean forest burnt 20 years ago. On the contrary, ephemeral species reduced their fitness where perennial beneficiary species increased their size. In our study system, *Ononis* has a double ecological role as a very early colonizer on gypsum outcrops and a bridging species between early and late plant communities. This strengthens the key role that facilitation may be playing in the community and ecosystem assembly under severe stress conditions as defended by Bruno, Stachowicz & Bertness (2003), Callaway (2007) or Soliveres, Smit & Maestre (2015) and brings closer the temporal and spatial scales at which this process occurs. Finally, the estimation of the time frame for the establishment and development of facilitative interactions between a nurse and its potential beneficiary species, as performed here, may have ecological applications in restoration practice in a double way: first, by providing tools for a better choice of target species for restoration of facilitation-driven ecosystems, and second, by improving the selection of the microsite of plantation depending on the functional traits of selected species.

Acknowledgements

This study was supported by the BBVA Foundation (I Convocatoria de Ayudas de la Fundación BBVA a Proyectos de Investigación: Área de Ecología y Biología de la Conservación), the Spanish Ministerio de Economía y Competitividad (R+D Project CGL2014-58333-P) and the Research Grant 4742-5780 awarded to J.A.N. by the British Ecological Society. MG acknowledges support by the Programa Ramón y Cajal (Ministerio de Economía y Competitividad). Suggestions by the Associate Editor and two anonymous referees are gratefully acknowledged.

Data accessibility

All data used in this manuscript are present in the manuscript and its Supporting Information.

References

Bertness, M.D. & Callaway, R. (1994) Positive interactions in communities. *Trends in Ecology & Evolution*, **9**, 191–193.

Brooker, R.W., Maestre, F.T., Callaway, R.M., Lortie, C.L., Cavieres, L., Kunstler, G. *et al.* (2008) Facilitation in plant communities: the past, the present and the future. *Journal of Ecology*, **96**, 18–34.

Bruno, J.F., Stachowicz, J.J. & Bertness, M.D. (2003) Inclusion of facilitation into ecological theory. *Trends in Ecology and Evolution*, **18**, 119–125.

Butterfield, B.J. & Briggs, J.M. (2011) Regeneration niche differentiates functional strategies of desert woody plant species. *Oecologia*, **165**, 477–487.

Butterfield, B.J. & Callaway, R.M. (2013) A functional comparative approach to facilitation and its context dependence. *Functional Ecology*, **27**, 907–917.

Callaway, R.M. (2007) *Positive Interactions and Interdependence in Plant Communities*. Springer, Dordrecht.

Carrick, P.J. (2003) Competitive and facilitative relationships among three shrub species, and the role of browsing intensity and rooting depth in the Succulent Karoo, South Africa. *Journal of Vegetation Science*, **14**, 761–772.

Castillo, J.P., Verdú, M. & Valiente-Banuet, A. (2010) Neighborhood phylodiversity affects plant performance. *Ecology*, **91**, 3656–3663.

Caturla, R.N., Raventós, J., Guárdia, R. & Vallejo, V.R. (2000) Early post-fire regeneration Dynamics of *Brachypodium retusum* Pers. (Beauv.) in old fields of the Valencia region (eastern Spain). *Acta Oecologica*, **21**, 1–12.

Cornelissen, J.H.C., Lavorel, S., Garnier, E., Díaz, S., Buchmann, N., Gurvich, D.E. *et al.* (2003) Handbook of protocols for standardised and easy measurements of plant functional traits worldwide. *Australian Journal of Botany*, **51**, 335–380.

Crawley, M.J. (2005) *Statistics: An Introduction Using R*. John Wiley & Sons Ltd., Chichester, UK.

De Baets, S., Poesen, J., Knapen, A., Barberá, G.G. & Navarro, J.A. (2007) Root characteristics of representative Mediterranean plant species and their erosion-reducing potential during concentrated runoff. *Plant and Soil*, **294**, 169–183.

De Luis, M., Raventós, J., Cortina, J., González-Hidalgo, J.C. & Sánchez, J.R. (2004) Fire and torrential rainfall: effects on the perennial grass *Brachypodium retusum*. *Plant Ecology*, **173**, 225–232.

Díaz, S. & Cabildo, M. (1997) Plant functional types and ecosystem function in relation to global change. *Journal of Vegetation Science*, **8**, 463–474.

Goberna, M., Pascual, J.A., García, C. & Sánchez, J. (2007) Do plant clumps constitute microbial hotspots in semiarid Mediterranean patchy landscapes? *Soil Biology and Biochemistry*, **39**, 1047–1054.

Guerrero-Campo, J., Palacio, S., Pérez-Rontomé, C. & Montserrat-Martí, G. (2006) Effect of root system morphology on root-sprouting and shoot-rooting abilities in 123 plant species from eroded lands in North-east Spain. *Annals of Botany*, **98**, 439–447.

Herrera, C.M. (1992) Historical effects and sorting processes as explanations for contemporary ecological patterns: character syndromes in Mediterranean woody plants. *American Naturalist*, **140**, 421–446.

Joffre, R., Rambal, S. & Damesin, C. (1999) Functional attributes in Mediterranean-type ecosystems. *Handbook of Functional Plant Ecology* (eds F.I. Pugnaire & F. Valladares), pp. 347–380. Marcel Dekker, New York, NY, USA.

Kawai, T. & Tokeshi, M. (2007) Testing the facilitation-competition paradigm under the stress-gradient hypothesis: decoupling multiple stress factors. *Proceedings of the Royal Society B*, **274**, 2503–2508.

Kellman, M. & Kading, M. (1992) Facilitation of tree seedling establishment in a sand dune succession. *Journal of Vegetation Science*, **3**, 679–688.

Le Roux, P.C., Shaw, J.D. & Chown, S.L. (2013) Ontogenetic shifts in plant interactions vary with environmental severity and affect population structure. *New Phytologist*, **200**, 241–250.

Lloret, F., Peñuelas, J. & Estiarte, M. (2005) Effects of vegetation canopy and climate on seedling establishment in Mediterranean shrubland. *Journal of Vegetation Science*, **16**, 67–76.

Maestre, F.T. & Cortina, J. (2004) Do positive interactions increase with abiotic stress? A test from a semi-arid steppe. *Proceedings of the Royal Society of London B: Biological Sciences*, **271**(Suppl), S331–S333.

Maestre, F.T., Callaway, R.M., Valladares, F. & Lortie, C.J. (2009) Refining the stress-gradient hypothesis for competition and facilitation in plant communities. *Journal of Ecology*, **97**, 199–205.

McIntire, E.J.B. & Fajardo, A. (2014) Facilitation as a ubiquitous driver of biodiversity. *New Phytologist*, **201**, 403–416.

Merlo, M.E., Mota, J.F. & Sánchez-Gómez, P. (2011) Ecofisiología y adaptaciones de las plantas vasculares a las características físicas y químicas de sustratos especiales. *Diversidad vegetal de las yeseras ibéricas. El reto de los archipiélagos edáficos para la biología de la conservación* (eds J.F. Mota, P. Sánchez-Gómez & J.S. Guirado), pp. 53–73. ADIF-Mediterráneo Asesores Consultores, Almería.

Michalet, R., Brooker, R.W., Cavieres, L.A., Kikvidze, Z., Lortie, C.J., Pugnaire, F.I. *et al.* (2006) Do biotic interactions shape both sides of the humped-back model of species richness in plant communities? *Ecology Letters*, **9**, 767–773.

Miriti, M.N. (2006) Ontogenetic shift from facilitation to competition in a desert shrub. *Journal of Ecology*, **94**, 973–979.

Moreno-Gutiérrez, C., Dawson, T.E., Nicolás, E. & Querejeta, J.I. (2012) Isotopes reveal contrasting water use strategies among coexisting plant species in a Mediterranean ecosystem. *New Phytologist*, **196**, 489–496.

Mota, J.F., Sánchez-Gómez, P. & Guirado, J.S. (2011) *Diversidad vegetal de las yeseras ibéricas. El reto de los archipiélagos edáficos para la biología de la conservación*. ADIF-Mediterráneo Asesores Consultores, Almería.

Navarro-Cano, J.A. (2008) Effect of grass litter on seedling recruitment of the critically endangered *Cistus heterophyllus* in Spain. *Flora-Morphology, Distribution, Functional Ecology of Plants*, **203**, 663–668.

Navarro-Cano, J.A., Goberna, M., Valiente-Banuet, A., Montesinos-Navarro, A., García, C. & Verdú, M. (2014) Plant phylodiversity enhances soil microbial productivity in facilitation-driven communities. *Oecologia*, **174**, 909–920.

Navarro-Cano, J.A., Verdú, M., García, C. & Goberna, M. (2015) What nurse shrubs can do for barren soils: rapid productivity shifts associated with a 40 years ontogenetic gradient. *Plant and Soil*, **388**, 197–209.

Pueyo, Y., Alados, C.L., Maestro, M. & Komac, B. (2011) Gypsophile vegetation patterns under a range of soil properties induced by topographical position. *Plant Ecology*, **189**, 301–311.

Pugnaire, F.I. & Lazaro, R. (2000) Seed bank and understorey species composition in a semi-arid environment: the effect of shrub age and rainfall. *Annals of Botany*, **86**, 807–813.

Pugnaire, F.I., Haase, P., Puigdefábregas, J., Cueto, M., Clark, S.C. & Incoll, D. (1996) Facilitation and succession under the canopy of a leguminous shrub, *Retama sphaerocarpa*, in a semi-arid environment in south-east Spain. *Oikos*, **76**, 455–464.

R Core Team (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna. Available from: http://www.R-project.org/.

Shmida, A. & Whittaker, R.H. (1981) Pattern and biological microsite effects in two shrub communities, southern California. *Ecology*, **62**, 234–251.

Siles, G., Rey, P.J., Alcántara, J.M. & Ramírez, J.M. (2008) Assessing the long-term contribution of nurse plants to restoration of Mediterranean forests through Markovian models. *Journal of Applied Ecology*, **45**, 1790–1798.

Soliveres, S., Smit, C. & Maestre, F.T. (2015) Moving forward on facilitation research: response to changing environments and effects on the diversity, functioning and evolution of plant communities. *Biological Reviews*, **90**, 297–313.

Soliveres, S., DeSoto, L., Maestre, F.T. & Olano, J.M. (2010) Spatio-temporal heterogeneity in abiotic factors modulate multiple ontogenetic shifts between competition and facilitation. *Perspectives in Plant Ecology, Evolution and Systematics*, **12**, 227–234.

Suzuki, N., Rivero, R.M., Shulaev, V., Blumwald, E. & Mittler, R. (2014) Abiotic and biotic stress combinations. *New Phytologist*, **203**, 32–43.

Tewksbury, J.J. & Lloyd, J.D. (2001) Positive interactions under nurse-plants: spatial scale, stress gradients and benefactors size. *Oecologia*, **127**, 425–434.

Tielbörger, K. & Kadmon, R. (2000) Temporal environmental variation tips the balance between facilitation and interference in desert plants. *Ecology*, **81**, 1544–1553.

Valiente-Banuet, A. & Verdú, M. (2008) Temporal shifts from facilitation to competition occur between closely related taxa. *Journal of Ecology*, **96**, 489–494.

Valiente-Banuet, A. & Verdú, M. (2013) Plant facilitation and phylogenetics. *Annual Review of Ecology, Evolution and Systematics*, **44**, 347–366.

Valiente-Banuet, A., Vital, A., Verdú, M. & Callaway, R.M. (2006) Modern Quaternary plant lineages promote diversity through facilitation of ancient Tertiary lineages. *Proceedings of the National Academy of Sciences of the USA*, **103**, 16812–16817.

Received 22 September 2015; accepted 15 March 2016
Handling Editor: Laura Llorens

Supporting Information

Additional Supporting information may be found online in the supporting information tab for this article:

Fig. S1. Average monthly rainfall (±SD) for the period 1999–2012 (bars) and monthly rainfall from December 2012 to November 2013 (line diagram) in a climate station near the study area (data from http://siam.imida.es).

Fig. S2. Seedling emergence of the species tested as a function of *Ononis* age.

Appendix S1. Microclimatic, soil and plant data used in the analyses

Table S1. Data used to characterize the microenvironment beneath the nurse canopy.

Table S2. Seedling emergence of sown species.

Table S3. Presence of Quaternary and Tertiary species beneath *Ononis tridentata* plants.