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Females engage in stronger relationships: positive and negative effects of shrubs are more intense for *Poa ligularis* females than for males

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• **Background and Aims** Dioecious plants are of particular concern in view of global environmental changes because reproductive females are more sensitive to abiotic stresses, thus compromising population viability. Positive interactions with other plants may counteract the direct effects of any abiotic environmental stress, allowing them to thrive and maintain a viable population in suboptimal habitats, although this process has not been tested for dioecious species. Furthermore, almost no data are available on the outcome of such species interactions and their link with local spatial patterns and sex ratios.
• **Methods** We set up a field experiment with *Poa ligularis*, a dioecious native grass from the arid grasslands of South America. We studied the interaction of male and female plants with cushion shrubs of contrasting ecological strategies. We experimentally limited direct shrub–grass competition for soil moisture and transplanted plants to evaluate the amelioration of abiotic stress by shrub canopies (i.e. sun and wind) on grasses. We also studied the distribution of naturally established female and male plants to infer process–pattern relationships.
• **Key Results** Positive canopy effects as well as negative below-ground effects were more intense for females than for males. Deep-rooted *Mulinum spinosum* shrubs strongly facilitated survival, growth and reproduction of *P. ligularis* females. Naturally established female plants tended to distribute more closely to *Mulinum* than co-occurring males. Female growth suffered intensive negative root competition from the shallow-rooted *Senecio filaginoides* shrub.
• **Conclusions** Interactions with other plants may reduce or enhance the effect of abiotic stresses on the seemingly maladapted sex to arid environments. We found that these biased interactions are evident in the current organization of sexes in the field, confirming our experimental findings. Therefore, indirect effects of climate change on population sex ratios may be expected if benefactor species abundances are differentially affected.

Key words: Arid ecosystems, competition, dioecy, facilitation, female plants, male plants, *Mulinum spinosum*, Patagonian steppe, plant–plant interactions, *Poa ligularis*, positive interactions, *Senecio filaginoides*.

INTRODUCTION

It has been proposed that dioecious plant populations in harsh environments should be male-biased due to the higher susceptibility of female plants to abiotic stress (Dawson and Ehleringer, 1993; Delph, 1999; Obeso, 2002). However, the expected pattern of male preponderance in stressful habitats is not always found (Verdú and García-Fayos, 1998; Graff *et al.*, 2013; Juvany and Munné-Bosch, 2015; Moreno and Bertiller, 2016). While there are numerous physiological and genetic explanations for female-biased sex ratios in some populations (Field *et al.*, 2013), little attention has been given to the effect of biotic interactions on the performance of dioecious species (but see Verdu *et al.*, 2004; Montesinos *et al.*, 2007; Lortie and Reid, 2012; Graff *et al.*, 2013). Plant–plant interactions (facilitation and exploitation competition) may buffer or increase the stress experienced within a site and largely drive population vital rates (Goldberg, 1990; Liancourt *et al.*, 2013; López *et al.*, 2016), and thus sex ratios (Graff *et al.*, 2013).

In harsh environments, microhabitat differences may allow dioecious species to maximize fitness by restricting more stress-sensitive females to low-stress microsites and allowing males to stock resource-poorer microsites. Shrubs are frequently responsible for the microhabitat heterogeneity in sparsely vegetated arid lands (Noy-Meir, 1973; Aguiar and Sala, 1999; Yang *et al.*, 2015). Some studies have documented the spatial segregation of sexes (Bierzychudek and Eckhart, 1988) associated with the microhabitat heterogeneity generated by shrub patches. Females were found to be distributed close to shrubby patches, whereas males were more common in open areas (Delph, 1999; Bertiller *et al.*, 2000, 2002). Although not emphasized in the literature, this pattern of spatial segregation could be interpreted as a sex-biased facilitation of shrubs for females via habitat amelioration (but see Moreno and Bertiller, 2016). By providing shelter from wind and from extreme temperatures and by increasing soil fertility, facilitation mediated by shrubs could allow stress-sensitive females to grow and persist in environments where they would otherwise be limited (Palmer and Dixon, 1990; Bruno *et al.*, 2003). In this situation, the population sex ratio expected based on aridity could be different from that expected based on shrub cover (Moreno and Bertiller, 2016).

If species interactions are key drivers of the spatial pattern, the net balance should influence vital rates and the strength of the spatial segregation of sexes (Graff *et al.*, 2013). Morphological variation in the canopy and root architecture among dominant overstorey plants can determine understorey microenvironment as well as the magnitude of the interactive effects of canopy facilitation and below-ground interference (Callaway *et al.*, 1991; Michalet *et al.*, 2011; Schöb *et al.*, 2013; Aschehoug and Callaway, 2014; Bonanomi *et al.*, 2016). In systems where wind-driven desiccation is an important environmental stress factor for plant growth (Lambers *et al.*, 2008), compact and tall shrubs provided a better wind shield than smaller shrubs with loose canopies, thereby reducing evaporation (Schöb *et al.*, 2013). However, plants growing under dense canopies could also have the cost of reduced light (Holmgren *et al.*, 1997). Therefore, the level of shading created by nurse plants could determine a lack of a positive influence of their canopy, despite the improvements in water conditions (Reisman-Berman, 2007; Michalet *et al.*, 2011; Holmgren *et al.*, 2012). By contrast, below-ground competition is also expected to be intense in water limited systems, increasing the stress experienced by neighbouring species (Valladares and Pearcy, 2002; Michalet, 2007; Maestre *et al.*, 2009; Michalet *et al.*, 2016), and despite a canopy effect, root architecture could determine the intensity of the competition between woody and herbaceous growth forms (Callaway *et al.*, 1991; Bucci *et al.*, 2009; Aschehoug and Callaway, 2014; Semchenko *et al.*, 2017). Because of sex -differences in growth, survival, reproductive output, resource allocation and physiology (Dawson and Bliss, 1989), it might be expected that the genders will perform differently in facilitative/competitive interactions with other species (Verdu *et al.*, 2004; Montesinos *et al.*, 2007; Graff *et al.*, 2013). Therefore, the performance of dioecious species could increase or decrease depending on the interacting overstorey shrub species, and eventually this differential performance could result in a local spatial segregation of the sexes.

We studied the interaction of adult male and female plants of a dioecious grass species with neighbouring shrub species having morphological variation in root architecture and contrasting ecological strategies (Graff and Aguiar, 2017). The shrubs selected were hemispherical, with closed and tall canopies, but one species was deep-rooted and the other was shallow-rooted. Therefore, we expected that both shrubs would be canopy facilitators, but we also expected that soil effects of shallow-rooted species would impact more negatively on the performance of grasses than those of deep-rooted species because woody and herbaceous growth forms frequently compete for resources in the upper soil layers (Davis *et al.*, 1998; Bucci *et al.*, 2009). Through transplanting male and female plants at different distances of shrub species and experimentally reducing root competition, we tested the potential effects of plant–plant interactions on vital rates of males and females. We studied the distribution of female and male naturally established plants at this ecological scale to confirm the importance of the interaction balance (Graff and Aguiar, 2017).

MATERIALS AND METHODS

Study site

Field studies were conducted in the Patagonian steppe in south-western Chubut, Argentina (45°41′S, 70°16′W, 500 m a.s.l.). The study site was located within a 150-km^2 area at the Río Mayo Experimental Field Station (Instituto Nacional de Tecnología Agropecuaria). This community is co-dominated by few grasses and shrubs (only three species each), which contribute more than 96 % of the total plant cover and plant biomass (Fernandez *et al.*, 1991). Forbs contribute <1 % to plant cover. The vegetation is distributed in a two-phase mosaic, shrubs and grasses forming high cover patches within a matrix of sparse tussock grasses. The climate is arid, with an intense summer drought (Paruelo *et al.*, 1988). Mean annual precipitation in the last 30 years (1984–2013) was 134 mm with a winter and early spring (May to September) rainy season. Average monthly temperatures range from 3 °C in winter to 16 °C in summer (1984–2013) (field meteorological station data http://anterior.inta.gov.ar/region/pas/sipas2/cmp/agromet/index.html# revised August, 2017). Strong, dry winds blow predominantly from west to east with high intensities throughout the year, averaging more than 20 km h^{-1} during the growing season. The scarce precipitation and its distribution during winter lead to a high summer water deficit. Therefore, stress during the growing season is mainly induced by the lack of below-ground resources (water and nutrients) and by above-ground non-resource factors (wind, temperature, etc.) (Beltrán, 1997). As in other arid ecosystems, stress factors also vary at the patch scale (Soriano and Sala, 1986; Aguiar and Sala, 1994). Wind speed is five times lower and evaporative demand two times lower near shrub patches than in bare soil areas (Soriano and Sala, 1986; Graff and Aguiar, 2017 *in* ecy1703-sup-0001-AppendixS1.docx), while at the same time, soil water content is significantly lower than in open spaces (Graff and Aguiar, 2017 *in* ecy1703-sup-0001-AppendixS1.docx). Therefore, amelioration of non-resource stress factors by shrub canopies is coupled with an increase in resource stress factors driven by the water consumption of the potential benefactor (Sala *et al.*, 1989; Valladares and Pearcy, 2002; Maestre and Cortina, 2004).

Species description

Shrub species. We selected two out of the three dominant species as potential benefactor species. Shrubs were *Mulinum spinosum* [=*Azorella prolifera* (Cav.) G.M. Plunkett & A.N. Nicolas] and *Senecio filaginoides*, De Candolle (hereafter *Mulinum* and *Senecio*, respectively). Together the two species account for 76 % of the shrub cover in the Occidental district in the Patagonian steppe (Fernandez *et al.*, 1991). *Mulinum* is a deciduous, hemispherical-cushion-form shrub, of 70 cm height. *Senecio* is an evergreen species small and globose shrub of 60 cm height (Fernandez *et al.*, 1991). Both shrub species have a tap root with long lateral roots growing outward, but in *Senecio*, the lateral roots grow close to the soil surface and previous physiological and water use studies on adult plants indicate that its activity overlaps substantially with grasses (Golluscio and Oesterheld, 2007; Bucci *et al.*, 2009). In *Mulinum* the lateral roots start to grow horizontally 15 cm below the soil surface (Fernández and Paruelo, 1988; Sala *et al.*, 1989; Bucci *et al.*, 2009) and we assume that this root architecture has a lower competition effect on herbaceous species.

Sexual morphs of the grass species. We studied *Poa ligularis* Nees. ap. Steudel (hereafter *Poa*), a dominant species in non-degraded Patagonian shrub–grass steppes. *Poa* is a

wind-pollinated and strictly dioecious bunchgrass (section Dioicopoa). The species is endemic to the Patagonian steppe and Pampa region in Argentina (www.floraargentina.edu.ar). Male (staminate) and female (pistillate) plants are dimorphic in their reproductive characters and the hairiness of the floret (www.floraargentina.edu.ar). They are also dimorphic in morpho-chemical traits related to herbivory and to the environmental variation induced by surrounding vegetation. Female morphs produce more phenolic compounds and are also less palatable to herbivores than males (see table 1 in Graff *et al*, 2013). Females associated with shrub patches are taller and have increased blade length and area than those in inter-patch areas (Moreno & Bertiller, 2016). On the other hand, females allocate less to the root system (i.e. less root biomass, new roots and root/shoot ratio) than males (see table 1 in Graff *et al*, 2013). A high investment in root biomass is necessary for drought resistance (Lambers *et al.*, 2008) and could help also to minimize the negative impact of sharing below-ground resources with neighbours (Cahill and Casper, 2000; Wang *et al.*, 2010; Semchenko *et al.*, 2013). Accordingly, below-ground competitive effects of other grass neighbours have been reported to be more intense on the biomass of female adult plants than on males (Graff *et al*, 2013). Previous studies with *Poa* indicated that sex expression is constant (Bertiller *et al.*, 2000, 2002; Graff *et al.*, 2013), and females distribute more closely to large shrub patches in the Patagonian Monte than males (Bertiller *et al.*, 2000, 2002).

Shrub–sexual morph interactions: field experiment

The effects of each shrub species on male and female *Poa* plants were addressed with a manipulative experiment with sex (female or male), shrub canopy effect (beneath shrub canopy or in open areas) and root effect (full or reduced root competition) arranged on a three-way factorial randomized complete block design. We selected six paddocks where sheep grazing was excluded during the experiment. Within each of these six grazing exclosures (blocks $n = 6$), we selected 16 *Mulinum* and 16 *Senecio* naturally established shrubs of similar height, basal diameter and far from other shrubs (>3 m), to apply the eight treatments with two subreplicates per block of each treatment combination. The eight treatments were the combination canopy × root effects for each sex.

In the previous spring–summer (November–January), *Poa* plants growing within long-term exclosures were sexed and marked in the field to enable collection in May 2010 (when the experiment began). The 96 male and 96 female transplants comprising the entire experiment were obtained by dividing *Poa* plants into five-tiller individual plants. Because *Poa* plants are perennial, we selected stem bases with green tillers coming from the tussock periphery to form the five-tiller individuals.

To isolate the shrub canopy effect (facilitation or not), transplants were placed at the edge of the shrub canopy (beneath shrub canopy), and 1 m away from the shrub edge (open areas), within the bare soil matrix and far from the influence of the shrub canopy. Transplants placed beneath shrub canopies were located east of shrubs, where wind speed is five times lower and evaporative demand is two times lower than those of exposed windward areas (Soriano and Sala, 1986; Aguiar and Sala, 1994). Therefore, facilitation via habitat amelioration is more likely to occur in eastern edges (Graff & Aguiar, 2017).

The root effect (full or reduced competition) was achieved by isolating (or not) transplant roots from the influence of shrubs. Half of the transplants had a reduced root effect (or reduced competition) treatment that was achieved by digging a hole of 0.20 m in diameter by 0.30 m deep, and fitting a fine mesh cloth in the pits, which functioned as a barrier to growth of roots of neighbouring plants under beneficiary target plants (Aguiar *et al.*, 1992; Graff *et al.*, 2007, 2013). The holes were filled with the extracted soil after removing roots and stones by sieving. Transplants with no mesh around the sieved soil core were those that did not receive a reduced root effect treatment. Large shrub patches are frequently encircled by a dense ring of grasses that could obscure shrub effects on target species (Aguiar and Sala, 1994), so before applying the treatments, we removed the surrounding grasses to isolate the effect of the shrub on *Poa* plants.

We monitored transplant survival during two growing seasons. Alive transplants were harvested in January 2012 by digging pits 0.20 m in diameter by 0.3 m deep where the transplants were located. Vegetative (shoot and root) and reproductive (panicles) material was separated, oven dried at 70 °C for 48 h and weighed. Roots were separated from sandy soils by flotation and sieving (0.2-mm mesh sieve). Shrub effects on transplant survival were analysed for each shrub species separately. We used the GENMOD procedure in SAS (SAS Institute, 1996). The model assumed a binomial distribution and used a logit link function because the response variable was binary (lived or not).

We analysed the effect of each shrub species on *Poa* plants through an RCB three-way factorial ANOVA in SAS (SAS Institute, 1996). The response variable was total vegetative biomass (roots and shoots) and panicle number. Panicle biomass was not included because some seeds from female plants were dispersed before we harvested the plants. We had three crossed factors: sex (S) (males and females), distance to neighbour (D) (close and far) and barrier to root competition (B) (with or without). Transformed data of total vegetative biomass (ln) and panicle number (sqrt) were used to meet the assumption of homoscedasticity in ANOVA. We averaged the two subreplicates per block for statistical analyses. This resulted in six true replicates ($n = 6$).

We also evaluated the predicted positive, negative and net effects of shrubs on sexes by calculating the Relative Interaction Index (RII;Armas *et al.*, 2004) per block. Response variables were survival, total vegetative biomass at harvest and panicle number at harvest.

$$\mathrm{RII}_{\text{net effects}} : \left(\mathrm{CB}^{-} - \mathrm{FB}^{+}\right) / \left(\mathrm{CB}^{-} + \mathrm{FB}^{+}\right),$$

where C is close to shrub canopy, F is far from shrubs, and B is the barrier to root competition treatment (+ and − superscripts indicate with or without the mesh cloth, respectively). RII is symmetrical around 0 and ranges from +1 to − 1. We constructed the 95 % confidence intervals from the RII values obtained per block. Positive values indicate facilitation, negative values indicate competition and values not significantly different from zero indicate neutral/no effects (Armas *et al.*, 2004). We tested whether RII values differ from zero using a *t*-test for single means.

Shrub–sexual morph distribution: descriptive study

To complement the field experiment, we included an assessment of the cumulative frequency distribution of males and females in relation to the distance from shrub canopies measured during the flowering season. We used four long-term exclosures to sheep grazing and other large herbivores (settled in 1956, 1972, 1983 and 1994, respectively) to minimize the influence of large herbivores on the spatial distribution of plants. In each of the four exclosures, we selected shrubs of modal size along an imaginary line forming ten pairs of single plants of *Mulinum–Mulinum* and ten *Senecio–Senecio* shrubs. Selections were based on the criteria that the distance between the edge of each shrub was 2 m (which is approximately the maximum distance between shrub pairs found in exclosures; Cipriotti and Aguiar, 2010). Transects were placed from the edge of one shrub to the half distance of the other shrub edge to fully characterize the zone of influence of only one shrub on the distribution of *Poa* plants. At each transect, male and female distance to a focal shrub was recorded. We merged the data points from the ten transects per shrub per exclosure and then determined cumulative frequency distributions of the number of *Poa* plants at distances from shrubs. The distributions of cumulative frequencies of sexual morphs occurrence at varying distances from *Mulinum* or *Senecio* edges were compared using Kolmogorov–Smirnov tests. We also calculated the sex-ratio in the long-term exclosures. In each exclosure we established 50 × 1-m linear transect and we counted the total number of female and male plants.

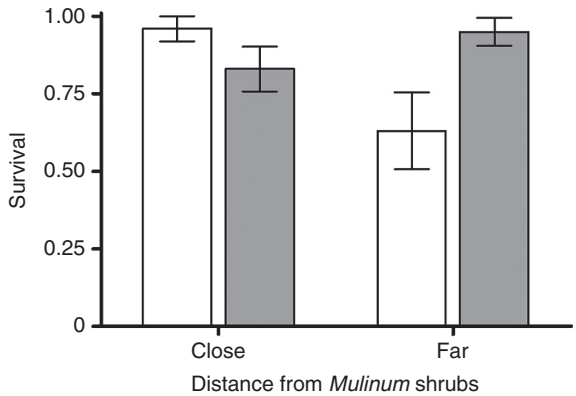

FIG. 1. Sex-biased survival response of sexual morphs of *Poa ligularis* to *Mulinum spinosum* shrubs. Proportion of female (white bars) and male (grey bars) plants that survived growing beneath *Mulinum* canopies (Close) and in open areas (Far) (Sex × Distance, $P = 0.0076$).

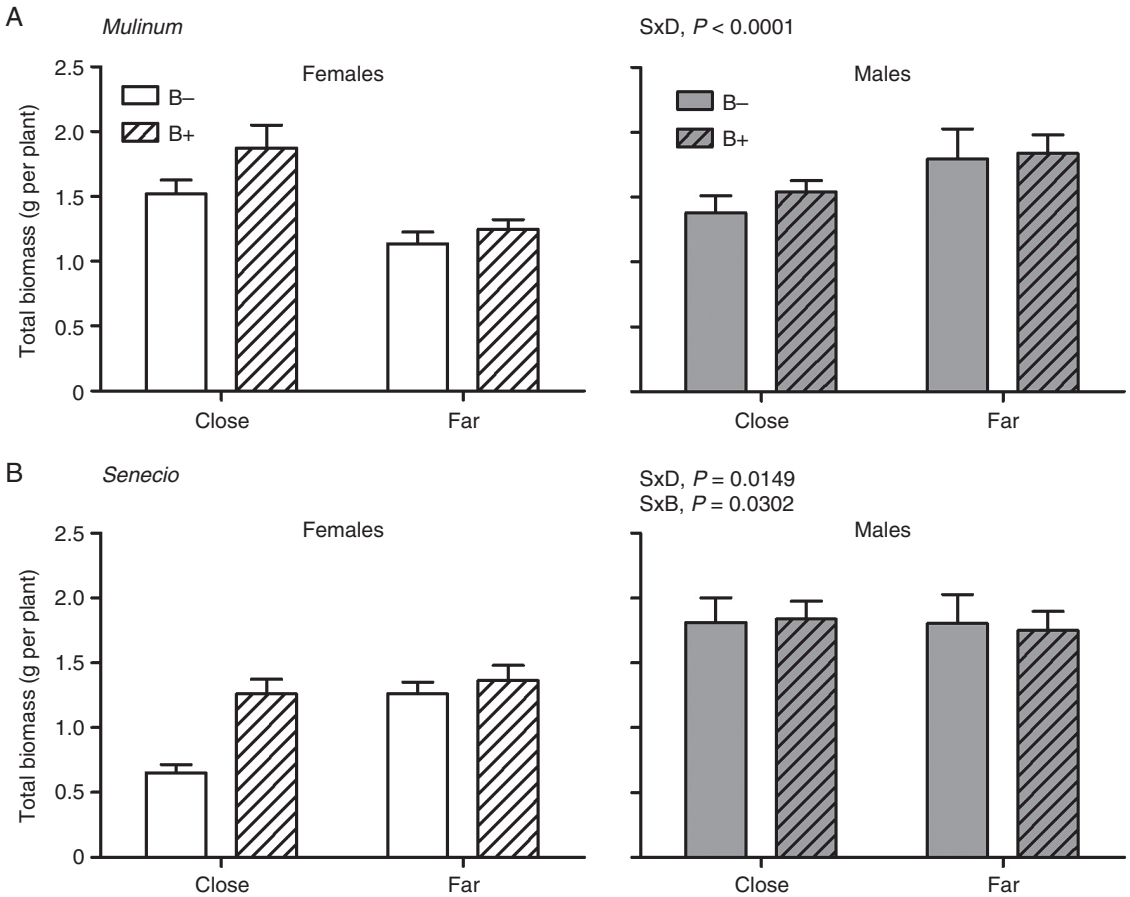

FIG. 2. Sex-biased growth response of sexual morphs of *Poa ligularis* to the interaction with shrubs. Total biomass at harvest of female (white bars) and male (grey bars) plants interacting with (A) *Mulinum spinosum* and (B) *Senecio filaginoides* shrubs. Plants grew in the close periphery to or far from potential nurses and with (B+) or without (B-) the barrier that reduces root competition on them. S, sex; D, distance from shrub; B, barrier factors.

RESULTS

Shrub–sexual morph interactions

A sex-biased survival of *Poa* plants was only detected in the interaction with *Mulinum* shrubs (Fig. 1). Females were more favoured by *Mulinum* canopies than males (Sex × Distance $P = 0.0076$). Almost all female plants survived when grown beneath *Mulinum* canopies, whereas survival of females was 37 % lower in open areas. Male survival was not affected by the distance to *Mulinum* canopies (Supplementary Data Table S1).

We found strong sex-related differences in total biomass at harvest in response to shrub species interactions (Fig. 2). While *Mulinum* canopies had positive effects on female total biomass, they had negative effects on male biomass (Sex × Distance, $P<0.0001$, Fig. 2A). Although plants tended to accumulate more biomass when shrub roots were excluded (Barrier, $P = 0.08$), this was a weak effect and no interaction with sexual morphs was detected (Sex × Barrier, $P = 0.64$) (Table S2).

Senecio shrubs did not affect the survival of female and male plants, but interacted with sexual morphs in an opposite way to that found for *Mulinum* (Fig. 2B). On average, females accumulated less biomass growing close to than far from *Senecio*, whereas males did not show differences in their biomass (Sex × Distance, $P = 0.0149$). Females and males were also affected differently by *Senecio* root competition (Fig. 2A) growing twice as large with the barrier (B+) than without it (B-) (Sex × Barrier, $P = 0.0302$). By contrast, males did not show differences in total biomass between the barrier treatments (Table S2).

A sex-biased response in panicle production was also detected in the interaction with shrubs (Fig. 3). In general, the patterns of response for reproduction were similar to those for plant biomass, but with different intensity depending on the shrub species. Females produced three times more reproductive structures close to *Mulinum* than far from them. By contrast, male panicle number was 40 % lower close to than far from the shrub (Sex × Distance, $P = 0.0002$), independently of the competition treatment. Similar but less significant results were found with *Senecio* (Sex × Distance, $P = 0.04$) (Table S3).

Net effects of shrubs were significant for female performance but not for male performance ($P < 0.05$). Whereas in the interaction with *Mulinum* the net effect on females was positive for survival, growth and panicle production, in the interaction with *Senecio* the balance was negative for growth (Fig. 4; Table S4).

Shrub–sexual morph distribution

A total of 217 *Poa* plants were recorded in the 80 transects placed from the edge of one shrub to the half distance of the

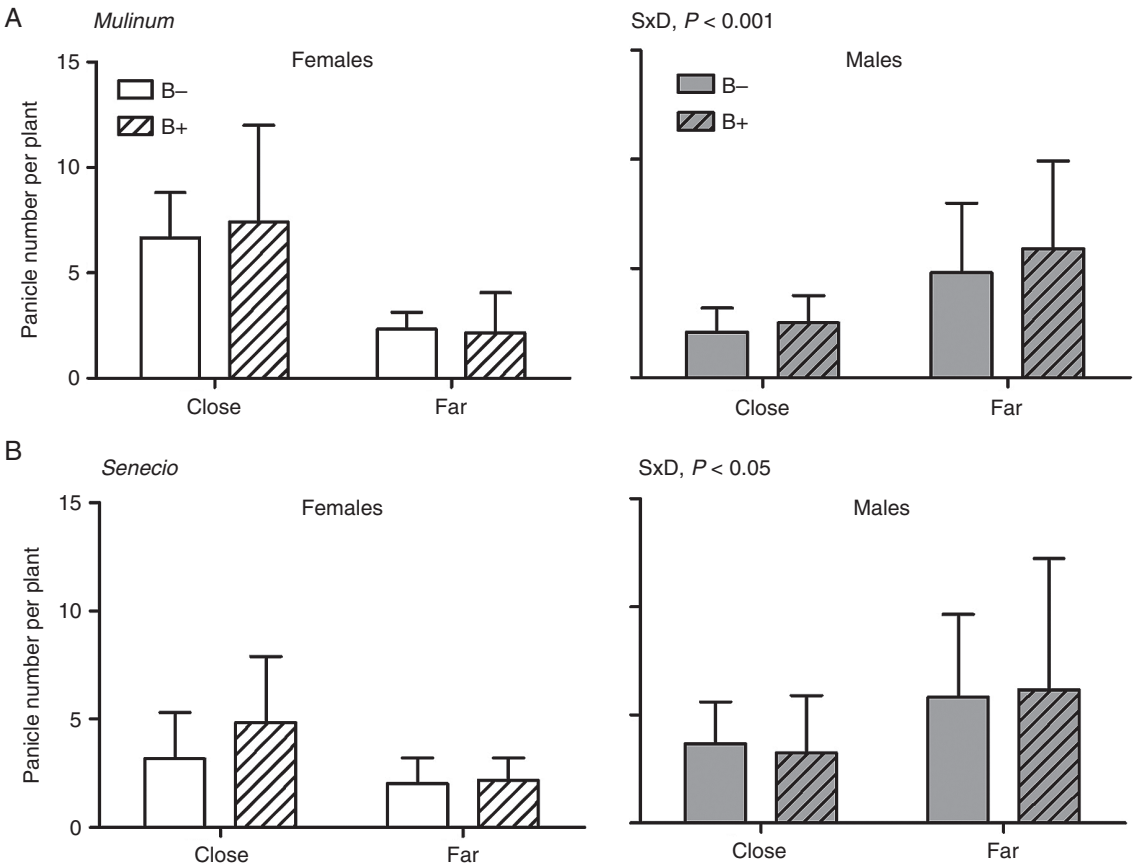

FIG. 3. Sex-biased panicle production of sexual morphs in response of *Poa ligularis* to the interaction with shrubs. Panicle number produced at the end of the second growing season of female (white bars) and male (grey bars) plants interacting with (A) *Mulinum spinosum* and (B) *Senecio filaginoides* shrubs. Plants grew in the close periphery to or far from potential nurses and with (B+) or without (B-) the barrier that reduces root competition on them. S, sex; D, distance from shrub; B, barrier factors.

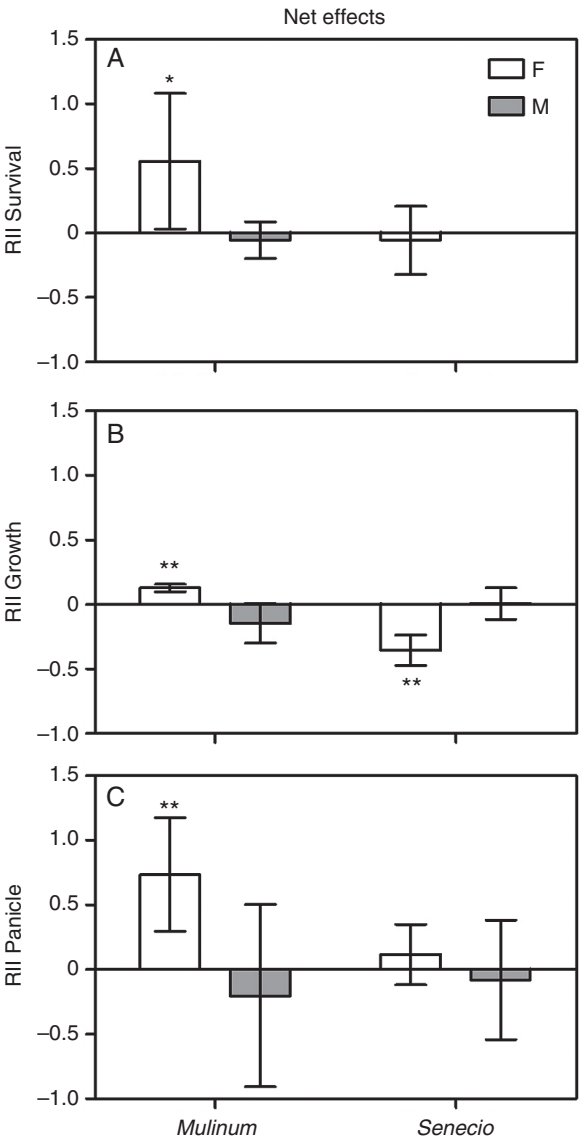

FIG. 4. Net interaction effects of shrubs on *Poa ligularis* females and males. Bars represent the Relative Interaction Index (RII; Armas *et al.*, 2004) of *Mulinum* and *Senecio* shrubs on each sexual morph. White bars for females and grey bars for males. Values are RII means and 95 % confidence intervals. Positive values indicate facilitation, negative values indicate competition and values not significantly different from zero indicate neutral/no effects (Cristina Armas *et al.*, 2004). F, females; M, males. Asterisks indicate significant deviation from zero value (one-sample *t*-tests): $*P < 0.05$, $**P < 0.01$. Calculations of the effects are explained in detail in the Methods.

other shrub edge, 111 in the *Mulinum–Mulinum* transects and 106 in the *Senecio–Senecio* transects. Females were distributed more closely to *Mulinum* shrub patches than males (Distance, 0.4122, $P = 0.002$), with a median of 40 and 65 cm, respectively (Fig. 5A). Females and males were distributed similarly from *Senecio* shrubs ($P = 0.677$), but no female individuals were found in close proximity of *Senecio* (up to 14 cm). The 5–10 % percentiles of female numbers were at 18.2–21 cm, whereas those for males were at 0–2.4 cm (Fig. 5B).

Sex ratio was female-biased and increased with exclosure age. Sex ratio was 2.2: 1, 1.7: 1, 1.3: 1 and 1.1: 1 in the exclosures settled in 1956, 1972, 1983 and 1994, respectively.

DISCUSSION

In dioecious plant species, population performance and viability can be projected in terms of reproductive females. Male-biased sex ratios then serve to lower the effective population size (N_e), which can lead to extinction (Hultine *et al.*, 2008, 2016). It has been proposed that positive interactions with other plant species may counteract the direct effects of the abiotic environment, allowing plants to thrive and maintain a viable population in suboptimal habitats (Hacker and Gaines, 1997; Bruno *et al.*, 2003), but this concept has not been explored for dioecious species. Our results confirmed that facilitation, mediated by shrub cover, could help females of dioecious plants to cope with environmental severity, but also that water depletion by neighbours may prevent females from benefitting from seemingly favourable conditions above ground. The current organization of sexes in the steppe environment confirmed that our experimental findings are important (*sensu* Tirado *et al.*, 2015) in the context of spatial and temporal variability controlling vital rates of plants in arid ecosystems.

Poa ligularis females interact with shrubs more strongly than males. Positive canopy effects as well as negative belowground effects were more intense for female vital rates than for male vital rates, and were also dependent on the shrub species. Canopies of *Mulinum* shrubs facilitated the survival, growth and reproduction of females, whereas there was a lack of a positive influence of *Senecio* canopies. The variation in the level of shade below both shrubs could explain the difference in their effect. Both shrubs have been described as hemispherical cushion-like shrubs, but with contrasting phenologies (Golluscio *et al.*, 2005). *Mulinum* is a deciduous species and *Senecio* is an evergreen species that intercepts more radiation than *Mulinum* in summer, when dry conditions are more marked (Graff and Aguiar, 2017). Previous studies have demonstrated that *Poa ligularis* increases chlorophyll content and photoprotective carotenoid concentration under drought (Cenzano *et al.*, 2013). Protection against high irradiance could be a cost in the shade rather than a benefit (Valladares, 2004; Valladares *et al.*, 2008). Therefore, the greater shade below *Senecio* canopies could partially explain the lack of a positive influence on *Poa* plants. Additionally, *Senecio*'s root competition was also strong on female growth. Negative root effects were more intense in the interaction with the shrub with the shallowest rooting depth. Overall net positive effects of *Mulinum*, the deep-rooted shrub, on females were also supported by the spatial organization in the field: naturally established female *Poa* plants tended to be distributed more closely to shrub edges than males. Therefore, based on the probably higher costs of reproduction (Graff *et al*, 2013), females of a dioecious grass in this arid environment were more strongly favoured by shrubs than males, but only when positive effects through habitat amelioration outweighed the negative effects of resource competition.

In anemophilous dioecious plants, it is frequent to find that sexes segregate by habitat, with the females being located in habitats of high quality and in the shelter of vegetation patches. By contrast, the males frequently grow in the more marginal zones or in more exposed sites with faster air currents (i.e. distant from a vegetation patch). The latter would enhance male function, by distributing their pollen and increasing opportunities for mating (Bierzychudek and Eckhart, 1988; Bertiller *et al.*, 2000).

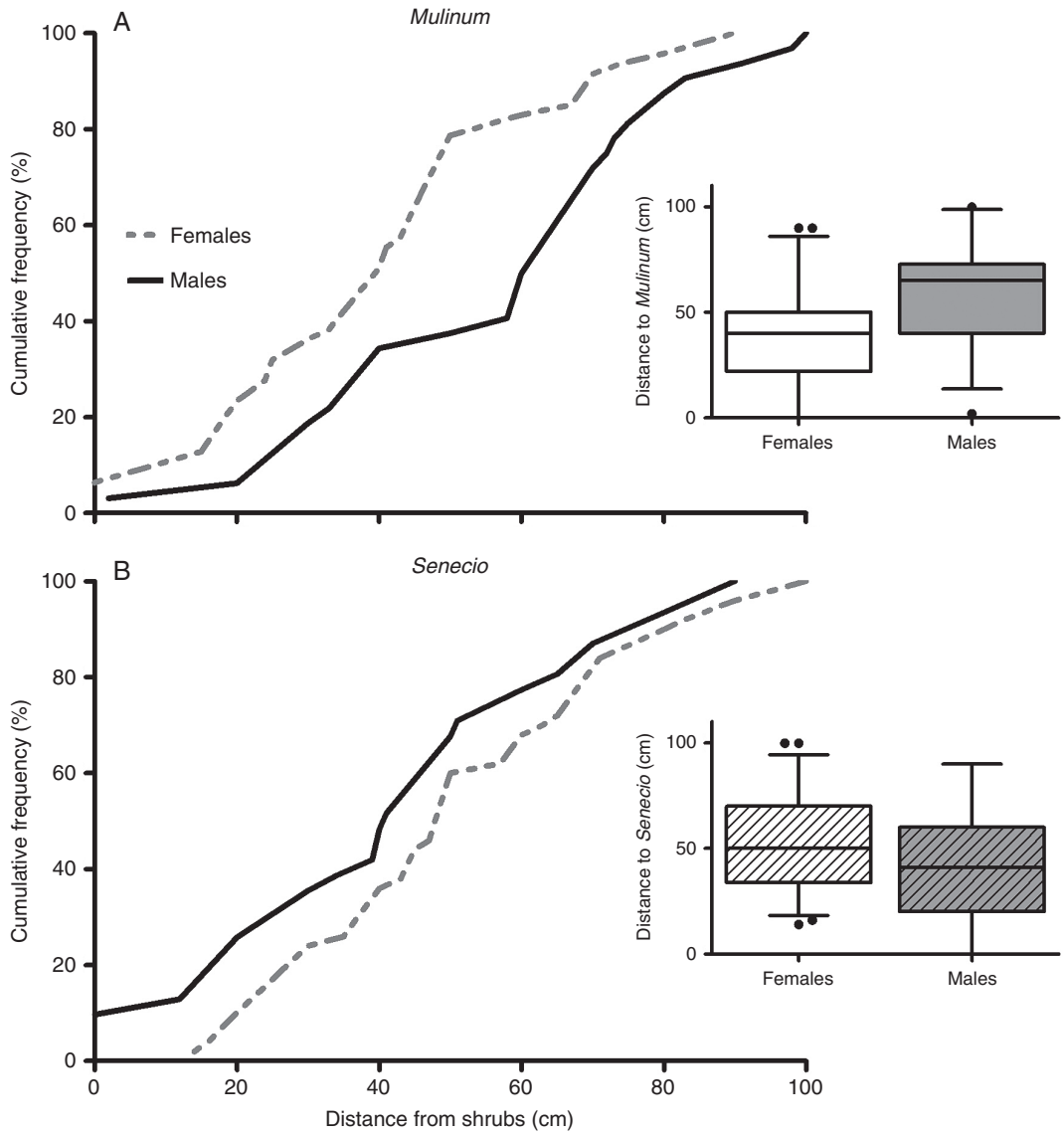

FIG. 5. Distribution of shrub–sexual morphs. Cumulative frequency of female (dotted curve) and male (solid line) plants at increasing distances from *Mulinum spinosum* (deciduous, deep-rooted) and *Senecio filaginoides* (evergreen and shallow-rooted) shrubs. Inset figures are box-and-whisker plots representing the 5–95 % percentile distribution and median values. Point outside whiskers are outliers.

For *Poa ligularis*, pollen production and dispersal occur in spring, after a humid winter season, and when soil water is not a constraint (Bertiller *et al.*, 2000). However, females complete fruiting at the beginning of the dry season (late spring to early summer). Thus, the reproductive effort of males can be maintained even in relatively more exposed sites (i.e. distant from a patch) than females. Confirming these ideas, we have found a consistent gender differential response on panicle production related to shrub distance. Females produce more panicles when close to rather than far from shrubs, whereas males showed the opposite pattern, suggesting a reproductive disadvantage in sheltered microsites for males (Dawson and Bliss, 1989; Delph, 1999).

Despite the prediction of a male preponderance in arid environments, previous studies in harsh environments did not confirm this pattern because of unexpected female-biased sex ratios (Graff *et al.*, 2013; Moreno and Bertiller, 2016). Furthermore, female numbers sometimes increase along regional environmental stress gradients (Gehring and Linhart, 1993; Moreno and Bertiller, 2016). The mechanisms responsible for the female-biased sex ratios observed in mature plant populations are not well understood and could be caused by both genetic and ecological factors (Field *et al.*, 2013). Our study sheds light on an unexplored factor that could drive the sex ratio of dioecious species in arid environments: facilitation. In general terms, facilitation enhances plant growth, reproduction and survival of understorey species, eventually expanding their realized niche (Bruno *et al.*, 2003). Facilitation can also explain some of the patterns in plant species interactions occurring in stressed ecosystems (Michalet, 2006). However, certain species are more likely to act as facilitators than others, depending on the ways the species influence their environment and resources (Callaway,

2007; Macek *et al.*, 2016). In our current experiment, performed in grazing exclosures, we found that only *Mulinum* shrubs exerted positive net effects on females. Furthermore, females generally grew closely associated with this species in the field. *Mulinum* is a palatable species and increases in numbers in exclosures to sheep grazing (Fernández *et al.*, 1992; Cipriotti and Aguiar, 2012). Current density is 0.31, 0.24 and 0.14 individuals m^{-2} in the exclosures settled in 1972, 1983 and 1994, respectively (M. R. Aguiar *et al*., unpubl. data). We propose that this indirect effect of grazers on benefactor density may explain the female-biased pattern previously found in exclosures (Graff *et al.*, 2013). Moreover, it could help to understand why female ratio increases with exclosure age in this study.

Our study and findings may have significance in predicting the potential response of dioecious species to global environmental changes. It has been proposed that populations of dioecious species will be directly threatened by an increase in aridity due to the higher susceptibility of female plants to abiotic stress (Hultine *et al*., 2008, 2016). Our study with two different shrub species supports the idea that the indirect effects of climate change may be as important as the direct effects. In general, climate change has been projected to increase aridity in temperate drylands worldwide during this century (Feng and Fu, 2013), but it will be coupled with a substantial increase in deep soil drought conditions during the growing season (Schlaepfer *et al.*, 2017), which may differentially affect woody plants according to their root architecture. In particular, stress-tolerant dominant species with shallow roots (Schlaepfer *et al.*, 2017), and species with thick evergreen leaves, low specific leaf area and high leaf dry matter content will be favoured (Gross *et al.*, 2013). This specialization of the species pool, expected under extreme conditions, will probably change the facilitation/competition balance towards an increase in the importance of competition as an assembly driver (McCluney *et al.*, 2012; Liancourt *et al.*, 2013; Siefert *et al.*, 2015). Climate change will thus decrease the reproductive success of many dioecious species by directly altering their individual fitness (Hultine *et al*., 2008, 2016), but also indirectly, by limiting the role of shrubs as benefactors of females, amplifying dioecious species loss.

CONCLUSIONS

Interactions with other plants may reduce or enhance the effect of abiotic stress on the seemingly maladapted sex to arid environments. We found that these biased interactions are evident in the current organization of sexes in the field, confirming our experimental findings. Therefore, indirect effects of climate change on population sex ratios may be expected if benefactor species abundances are differentially affected.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Table S1: Analysis of the survival response of *Poa ligularis*. Table S2: Analysis of the growth response of *Poa ligularis*. Table S3: Analysis of the panicle production of *Poa ligularis*. Table S4: Analysis of the net interaction outcomes among shrubs and *Poa ligularis* sexual morphs.

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