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Fire frequency effects on soil and pollinators: what shapes sexual plant reproduction?

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Abstract Increased fire frequency can significantly erode both soil properties and plant–pollinator interactions affecting plant reproductive success but they have seldom been assessed simultaneously. Here, we evaluate soil properties, pollinator assemblage and the reproductive success of two native Fabaceae herbs, *Desmodium uncinatum* and *Rhynchosia edulis*, growing in unburned, low and high fire frequency sites of Chaco Serrano across two consecutive years. *Desmodium uncinatum* is outcrossing with a high dependence on pollinators, whereas *R. edulis* is autogamous and completely independent of pollinators. We found that soil water content, nitrates and electrical conductivity significantly decreased in low and high fire frequency sites. Pollinator richness and composition visiting each plant species was similar across all fire frequency scenarios. However, fruit set of the exogamous *D. uncinatum* was strongly reduced in frequently burned sites, whereas fruit set of the autogamous *R. edulis* showed no significant changes. In both species, the probability of setting fruits was positively related to soil quality across fire frequency scenarios, implying that decreased reproduction was mainly driven by limitation of abiotic resources shaped by increased fire frequency. Because the pollinator-dependent *D. uncinatum* has a higher reproductive cost, reduced soil quality induced by fire frequency had stronger effects on its reproduction. Chronic reduction of sexual reproduction in frequently burned sites with depleted soils will limit population recruitment with negative consequences on long-term plant population persistence.

Keywords Chaco Serrano · Fire regime · Fruit set · Plant–pollinator interaction · Soil resources

Introduction

Fire represents an important disturbance factor shaping plant community structure and composition in many ecosystems throughout the world (Bond et al.

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2005). Plants are often adapted to a particular fire regime, which includes characteristic frequency, intensity and seasonality of fires through time (Keeley et al. 2011; Pausas 2015). However, human influences such as land use changes, global warming and livestock grazing can alter fire regimes by increasing or decreasing their frequency (Moritz et al. 2012) leading to a decline of plant species adapted to a specific fire regime and changing local plant community composition (Keeley et al. 2011). In the last decades, fire frequencies have increased in several ecosystems of South America such as the Amazon and the Chaco forests, as a result of anthropogenic activities (Bravo et al. 2010; Alencar et al. 2015). In a prospective view, it is expected that fire frequency will continue to increase substantially through this century in mid and high latitudes worldwide (Moritz et al. 2012). Particularly in central Argentina, the Sierras Chicas present the highest numbers of fire events, burned area, and fire frequency over the 1999–2011 period (Argañaraz et al. 2015). Both biophysical (increased precipitation, evapotranspiration and temperature seasonality) and anthropogenic factors (higher population density and closer urban areas) are currently the main determinants of the high frequency of fires in the region (Argañaraz 2016).

Increased fire frequency usually alters soil properties in different ways, affecting water availability and soil nutrient dynamics (Raison 1979). For example, frequently burned soils are typically less permeable to water infiltration, as a result of recurrent ash deposition that clogs soil pores and creates water-repellent layers (Certini 2005). In addition, frequent fires reduce or eliminate vegetation and litter, thus raindrops hit bared soils more strongly and water runoff events increase, further intensifying soil erosion (Certini 2005; González-Pelayo et al. 2015). Moreover, fire also alters soil chemical properties and its effects differ depending on both the time elapsed after fire and the fire frequency (Wan et al. 2001). Immediately after fire, organic carbon and nitrogen are partly volatilized to the atmosphere as a result of organic matter mineralization. Organic nitrogen can also mineralize to ammonium and nitrate forms (Certini 2005). However, while nitrate levels initially increase within the first year following a fire event, they consistently decrease afterwards due to lixiviation and absorption by mineral and organic matter (Wan et al. 2001; Certini 2005). As a result, fire increases soil nutrients in the short term, but high fire frequency depletes soil carbon and nitrogen in the long term (Certini 2005; Pellegrini et al. 2015).

Soil nutrient and water limitation triggered by frequent fires can have direct and indirect effects on sexual plant reproduction. Direct bottom-up effects of soil nutrients and water can shape plant reproductive output by controlling plant growth and/or flower and fruit production (Muñoz et al. 2005; Burkle and Irwin 2009, 2010; Brown and York 2016). Moreover, soil nutrient deficiency imposed by increased fire frequency can also indirectly limit seed and fruit set via decreased pollination attraction as a result of lowered flower abundance, flower display and/or reward production (nectar or pollen) (Burkle and Irwin 2010). Altogether, decreased abiotic resources coupled with reduced pollination will have the strongest effect on the reproduction of mostly outcrossing animal-pollinated plant species, which invest more in floral display and rewards because they are highly dependent on pollinator visitation to successfully set fruits (Harder and Aizen 2010; Schmid et al. 2015).

Fire can influence pollination process by triggering changes in pollinator population dynamics. Fire effects on pollinators have been increasingly studied over the last years and most studies have focused on the effects of time elapsed after fire (e.g., Potts et al. 2003; Brown et al. 2016a). Pollinator abundance such as bees drastically decreases immediately after fire due to increased mortality by exposure to lethal temperatures and destruction of nesting sites, but tends to recover rapidly thereafter and even to increase during the first year post-fire (Potts et al. 2003; Brown and York 2016). However, much less well studied are the indirect effects of fire frequency on pollinator fauna, which can occur at different scales. Not only plant richness may decrease at the community level (Carbone et al. 2017), but also plant individuals at the population level may modify the flower availability for pollinators in frequently burned sites (Van Nuland et al. 2013). Such changes in the quality and quantity of floral resources may affect pollinator visitation (e.g., Dafni et al. 2012; Lazarina et al. 2017). Because most angiosperms rely on animal pollinators for sexual reproduction (Ollerton et al. 2011), increased fire frequency can significantly modify pollination service (Winfree et al. 2009) negatively impacting plant reproduction and thus on the long-term persistence of plant populations (Aguilar et al.

2006; McKechnie and Sargent 2013). Particularly, limitation of pollen and/or pollinators will mostly affect the reproductive output of pollinator-dependent plants such as exogamous or strictly self-incompatible species (Campbell and Halama 1993; Burkle and Irwin 2009; McKechnie and Sargent 2013) as compared to self-compatible species that can set seeds via autogamy. In short, understanding fire frequency effects on abiotic resources and on pollinators and their interactions with plants should improve our predictions of plant reproductive responses in frequently burned sites.

Here, we assess soil properties, plant–pollinator interaction and reproduction of two native Fabaceae herbs, *Desmodium uncinatum* and *Rhynchosia edulis,* in unburned, low, and high fire frequency scenarios. These plant species are native, dominant herbs of the Chaco Serrano (Giorgis et al. 2011) and present contrasting breeding systems: *D. uncinatum* is mainly outcrossing and thus has an essential dependence on pollinators (Alemán et al. 2014), whereas *R. edulis* is autogamous having a null dependence on pollinators (Carbone 2017). We hypothesize that increased fire frequency erodes soil quality and also decreases floral abundance of these focal plants for insect pollinators. As a consequence, plant individuals growing in frequently burned sites will have reduced reproductive output as a result of both reduced soil resources and decreased pollinator visitation. Due to potential additive abiotic and biotic drivers, decreased reproductive output will be more pronounced in *D. uncinatum*, which has a higher reproductive dependence on pollinators.

Methods

Study system and species

The study area corresponds to the Chaco Serrano district, within the Chaco phytogeographical province (Cabrera 1971). The typical vegetation is a xerophytic subtropical forest, currently formed by compound mosaics of forest, scrubland and grassland as a consequence of human land use changes and recurrent fires (Luti et al. 1979; Gavier and Bucher 2004). The tree layer is dominated by *Lithraea molleoides*, *Acacia caven* and *Zanthoxylum coco* (Cabrera 1971). Elevation ranges from 400 to 1300 m and the climate is characterized as a warm continental type. The average precipitation is 900 mm per year, with a considerable variability between years (600–1500 mm, Malanca family pers. com.; Giorgis et al. 2013). Precipitation is concentrated in the warm season (October–March), delimiting a clear period of water deficit during autumn and winter. Wildfires typically coincide with the end of the dry season, when temperatures begin to increase, fuel moisture is low and winds reach their maximum speeds (Bravo et al. 2010). Fires in the Chaco Serrano are currently mainly caused by humans due to accidental or negligent ignitions. In the last decades, there has been a documented increase in fire frequency for the Chaco region, mainly associated with an increase in rains at a regional level (Bravo et al. 2010). The Sierras Chicas from central Argentina has been one of the most affected regions, with fire frequency estimated to be one fire every five to six years for 1999–2011 period (Argañaraz et al. 2015).

Desmodium uncinatum (Jacq.) DC. and *Rhynchosia edulis* Griseb. (Fabaceae, Papilionoideae) are perennial herbs native from North to South America that grow in mountainous regions. These species are a main component of the herbaceous stratum of shrublands and forests within Chaco Serrano region (Giorgis et al. 2011) and are abundant in communities with recurrent fires (Carbone and Aguilar 2016). Their underground reserve organs are woody rhizomes and xylopodial roots that represent a post-fire regeneration strategy allowing them to survive to fire and resprout quickly. However, these traits possess a limited potential for clonality (Clarke et al. 2013) and do not represent the main reproductive strategy as these Fabaceae can only reproduce by seeds. The growth season is spring and summer. Plants flower within the first year of growth and adults can live for several years (Carbone 2017). Both species have typical papilionate flowers and are mainly pollinated by bees (Hymenoptera), which visit their flowers searching for both nectar and pollen (*R. edulis*) or only pollen (*D. uncinatum*). In *D. uncinatum*, flowers are pink, of 1.5 cm in length and pluri-ovulate, whereas in *R. edulis* flowers are yellow, of less than 1 cm in length and bi-ovulated. Both species are self-compatible but they have contrasting reproductive strategies: *D. uncinatum* has an explosive pollination mechanism that is only activated after a pollinator visit. If the flower is not visited by a legitimate pollinator it cannot self-pollinate, thus it has a high dependence on pollinators (Alemán et al. 2014). Their flowers are mainly pollinated by social bumblebees (*Bombus* spp.) (Alemán et al. 2014, Carbone 2017). In contrast, *R. edulis* is visited mainly by solitary bees (Megachilidae) and can set fruits in the absence of pollinators via autonomous self-pollination, thus it has a low dependence on their solitary bee pollinators (Alemán et al. 2014; Carbone 2017).

Sampling design

Nine sites with different fire frequencies in the last 24 years (period 1991–2015) were selected using databases of fire records by Civil Defence of Rio Ceballos city (Giorgis et al. 2013) and Landsat TN and ETM satellite images (Argañaraz et al. 2015). These sites are located between 31°5′39″–31°9′12″S and 4°20′40″–4°24′11″W, covering an area of approximately 40 km^2. Sites were classified in three fire scenarios: three *unburned* sites; three *low fire frequency* sites with one to two fires and three *high fire frequency* sites with three to four fires (Online Resource 1). All sites were selected with the criteria of comparable elevation (820–1200 m), slope exposure (mainly N) and topographic position (middle hillside). All sites are located on private properties with similarly low stocking rates (bovine and equine cattle; Carbone et al. 2017). The unburned sites were represented by a physiognomy of open forest while low and high fire frequency sites showed a closed and open shrubland structure, respectively (Carbone et al. 2017). In each site, we marked 12 adult individuals of each species, totalling 108 individuals per species across the three contrasting fire frequency scenarios. All sampled individuals from each species were similar in size, and thereby of approximately similar age. Individuals were separated from each other by a minimal distance of three metres. We assessed pollination and reproduction across two consecutive reproductive periods 2013–2014 and 2014–2015. There was a marked difference in precipitation between these years measured in study area: 883.5 mm in 2013–2014 and 1154 mm in 2014–2015. Considering that the mean precipitation for 1990–2014 period is 882.5 mm (±179.3) for the study area (Carbone 2017), the period 2014–2015 is clearly above the overall mean precipitation of the area.

Soil properties

We collected four soil samples at each of the nine study sites in December 2013 as described in Carbone and Aguilar (2016). Soil samples (0–10 cm depth) were collected randomly around reproductive individuals of the studied species. Samples were placed in sealed plastic bags and stored in a portable Styrofoam cooler immediately after collection to prevent dehydration. Samples were taken directly to the laboratory and each of them was weighed and then placed in a drying oven at 60 °C until they reached a constant weight. Soil water content (%) was calculated as the ratio between water weight (g) and dry soil weight (g). After obtaining gravimetric soil moisture, the four subsamples from each site were mixed into a single composite sample and were taken to the Soil and Water Laboratory of the Faculty of Agronomic Sciences, National University of Córdoba. We analysed the following soil parameters: organic matter (OM, %), organic carbon (C, %), total nitrogen (N, %), C:N ratio, nitrates (NO^{3-}, ppm), extractable phosphorus (P, ppm), sulphates (SO_4^{2-}, ppm), current pH and electrical conductivity (EC, dS/m). The specific methodology used for each soil parameters is given in detailed in Carbone and Aguilar (2016).

Plant–pollinator interactions

Floral visitors of *D. uncinatum* and *R. edulis* were recorded in two flowering periods, December–March 2013–2014 and 2014–2015. We recorded all insects visiting open flowers for periods of approximately 15 min per individual plant (7–12 individuals per species per site) randomly across the entire flowering period. The overall sampling effort across the two periods was similar for each species totalling ca. 50 h of observation. We proceeded by two methods: direct focal observation and through a high-definition digital video camera. We conducted diurnal observations at moments of maximum floral display of each plant species at each site. Daily peak flowering in *D. uncinatum* occurred from 8:00 to 16:00 h, whereas for *R. edulis*, it was from 10:00 to 17:00 h. Observations were conducted randomly on plants at the various studied sites over the peak flowering time periods. In each observation, we counted the number of open flowers, the number of visited flowers and the taxonomic identity of each insect visitor. We distinguished legitimate pollinators from illegitimate floral visitor by observing contact with fertile floral parts, and the latter were not included in statistical analysis. Based on these observations, we estimated pollinator richness and the relative abundance of each pollinator species at each site, and defined the frequency of pollinator visits as the number of flower visits per individual plant per observation period (ca. 15 min). Pollinator observations were conducted on the same individuals we later measured reproductive traits.

Reproduction

We marked all inflorescences in each *D. uncinatum* individual and at least six inflorescences in *R. edulis* individuals in the two flowering periods (2013–2014 and 2014–2015). The measurements were conducted on the same individuals across the two flowering periods (10–12 individuals per species per site), except those that had died which were replaced by other adult plants of the population (1–2 individuals per species per site). We counted the number of flowers in each inflorescence and later the fruits produced by natural pollination from each marked inflorescence. Fruit set was calculated as number of mature fruits/number of marked flowers. In addition, we measured total flower production per individual plant across the two reproductive seasons in all fire frequency sites.

Data analysis

We assessed fire frequency effects on soil properties with permutational multivariate analysis of variance using a dissimilarity matrix with all variables of soil fertility previously mentioned obtained at the site level, which was performed with *adonis* function (999 permutations) of the *vegan* package (Oksanen et al. 2016). We also included the elevation of each study site (and their interaction with fire frequency) as an explanatory variable with the aim of incorporating the possible influence of the position of the study sites in the model. This analysis allows partitioning the variability of the multivariate dataset (soil properties) attributed to different explanatory variables, and uses permutation test to indicate their significances (Oksanen et al. 2016). In addition, to determine whether there were differences in the levels of each soil property among the three fire frequency scenarios, we used generalized linear models (*glm* function from the *stats* package) for variables with binomial error distribution (OM, C and N) and linear models with *F*-statistics for the rest of the variables that had normal error distribution.

We analysed the effects of fire frequency on pollinator richness with a non-asymptotic approach based on rarefaction and extrapolation of incidence data for standardized samples with a common sample completeness rather than size among fire frequency scenarios with iNEXT software (Chao et al. 2016). This methodology represents a unified sampling framework which allows performing fair and meaningful comparisons of species richness among incomplete samples. The pollinator richness and the 95% confidence interval were estimated for each condition at the same completeness with a resampling of 100 bootstraps (Chao et al. 2016). In addition, pollinator composition was compared among fire regimes through a one-way non-parametric similarity analysis, ANOSIM (999 permutations). For this analysis, we used a matrix with Jaccard similarity values based on the presence/absence data of pollinator species per site, using the *vegan* package (Oksanen et al. 2016). This analysis allows testing the null hypothesis that the composition of pollinator species is not different among fire frequency scenarios, using the *R* statistic as indicator of the degree of similarity between communities, with values near zero indicating completely identical composition (Oksanen et al. 2016).

To test the effects of fire frequency on pollinator visits and plant reproductive variables, we employed generalized linear mixed models (GLMM). We used fire frequency as the fixed effect with three levels (unburned, low fire frequency and high fire frequency). Site and period were used as random factors with nine and two levels, respectively. According to the error distribution of each response variable, we performed different GLMM extensions. Frequency of pollinator visits exhibited an excess of zero observations (no pollinators registered), which is usual for pollinator observation data with short-sampling intervals (Ebeling et al. 2008) and it is a problem that typically occurs in count datasets. Therefore, we performed a GLMM with a negative binomial family distribution and zero-inflation (*glmmadmb* function of the *glmmADMB* package). After checking assumptions for the inclusion of a covariate, we added the number of open flowers as a covariate in this model to test the effect of floral offer per plant on frequency of pollinator visits. Inclusion of fixed and random effects in the model was performed with analysis of deviance of nested models. Because random effects were not significant, we analysed visitation frequency using an extension of generalized linear models pooling data across sites and years: hurdle model (*hurdle* function of the *pscl* package), which in addition to overdispersion is capable of capturing excess of true zeros of frequency of visits (i.e., it assumes that zeros arise from a single process and not from sampling fails). These models have two components: a hurdle component models zero *vs*. larger counts (binomial) and a truncated count model (negative binomial) for positive counts (Zeileis et al. 2007). This approach is able to estimate the probability that a plant be visited and then estimates the relative mean number of pollinator visits between plants that were visited at least once. Significance of the fixed effect was performed with likelihood-ratio test of nested models. These analyses were performed separately for each species.

To evaluate fire frequency effects on plant reproductive variables, we used GLMM with negative binomial error distribution for total flower production because these count data had over-dispersion and binomial error distribution for fruit set (*glmer.nb* and *glmer* functions from the *lme4* package). For these analyses, the structure of fixed and random effects was the same as previously detailed. After checking assumptions and fit of model, significance of fixed effects was assessed with Wald-*Z* statistics. To evaluate period random effects (with different precipitations between 2013–2014 and 2014–2015) and site random effects, we compared nested models (one with and one without the random effects) with global model using a likelihood-ratio test (LRT). Model parameters were estimated with restricted maximum likelihood methods. Finally, to evaluate to what extent the variability observed in plant fitness correlates with soil quality, we tested for relationships between fruit set and an integrated soil quality variable (log-transformed) using a generalized linear model (GLM) with a binomial error distribution for each plant species. This analysis was performed only for 2013–2014 period, which was the period when soil samplings and plant reproductive traits were measured simultaneously (see Carbone and Aguilar 2016 for a similar approach with leaf traits). All analyses were performed in R 3.3.1.

Results

Soil properties

Fire frequency significantly explained variations in soil properties ($R^2 = 0.498$, $P = 0.034$), independently of elevation of the sites ($R^2 = 0.114$, $P = 0.199$; Online Resource 2). Soil samples from the unburned sites had significantly higher water content, nitrates and electrical conductivity as compared to the low and high fire frequency sites (Fig. 1). The rest of the soil parameters also showed higher values in the unburned sites; however, they were not significantly different among the three fire frequency conditions (Online Resource 3). Water content, nitrates and electrical conductivity were highly correlated among them (Pearson's r 0.72–0.88, $P < 0.0001$), thus we calculated an integrated soil quality variable by multiplying these three significant soil variables, following Aguirre-Acosta et al. (2014). This new integrated soil quality variable (log-transformed) was then used to analyse its relationship with fruit set in both plant species.

Plant–pollinator interactions

We observed a total of 14 insect species visiting the two plant species across the different fire frequency scenarios in both flowering periods but not all of them were effective pollinators (Table 1). All Diptera and Lepidoptera species observed as well as two Hymenoptera species (*Colletes* sp. and *Trimeria* sp.) were unable to activate the pollination mechanism in the flower of both legume species, thus were not effective pollinators (Table 1). The rest of the observed bee species were able to accomplish effective pollination at each visit and thus were the only species included in the pollinator assemblage analyses. These pollinator species can be split into two functional groups: one group of social bees mainly represented by genus *Bombus* (Apidae); and the other group of solitary bees such as *Megachile* sp. and *Notanthidium* sp. (Megachillidae), and carpenter bees (*Xylocopa ordinaria*).

Fire frequency did not significantly affect the richness of pollinator species of *D. uncinatum* and *R. edulis* in any of the two flowering periods, which is evidenced by the wide overlap of confidence intervals estimated for each fire frequency condition (Online Resource 4). Regarding the pollinator assemblage,

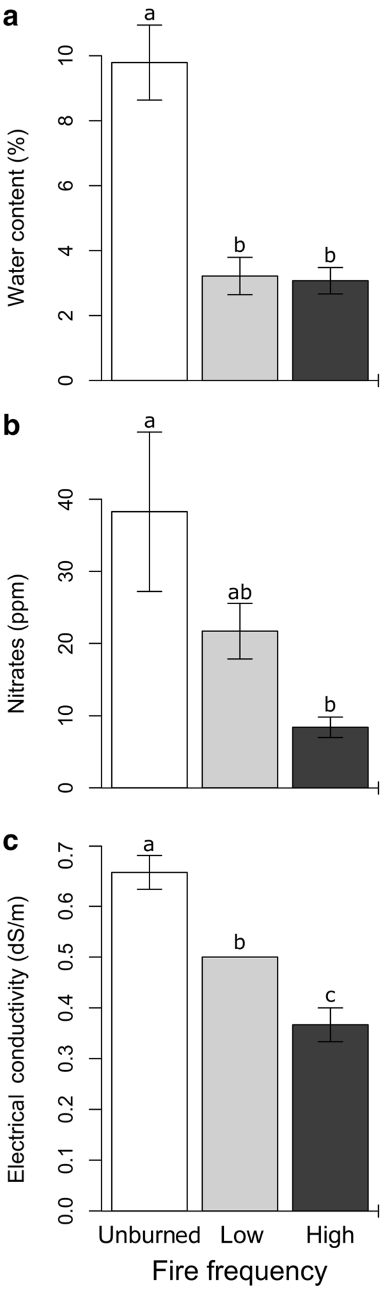

Fig. 1 Water content (**a**), nitrates (**b**) and electrical conductivity (**c**) of soils from three different fire frequencies: unburned, low (1–2 fires) and high fire frequency (3–4 fires) in the 1991–2015 period. Values are mean ± SD. Different letters indicate statistical significant differences ($P < 0.05$) in parameter value among fire frequency scenarios, according to GLM analysis

there were no differences in pollinators composition across the fire frequency scenarios for *D. uncinatum* (period 2013–2014: $R = 0.02$, $P = 0.44$; period 2014–2015: $R = -0.11$, $P = 0.77$) and *R. edulis* (period 2013–2014: $R = 0.02$, $P = 0.43$; period 2014–2015: $R = -0.09$, $P = 0.74$). The most abundant pollinators of *D. uncinatum* were bumble bees in all fire frequency scenarios, accounting for 40–84% of total visits, with *Bombus opifex* leading the visits followed by *B. bellicosus* (Table 1). In the three fire frequency scenarios, *R. edulis* was mainly visited by the solitary bees, specifically by Megachilidae species in unburned and low fire frequency sites and by *X. ordinaria* in high fire frequency condition (Table 1).

The number of pollinator visits in *D. uncinatum* was significantly explained by fire frequency and flower number as a covariate, while pollinator visits in *R. edulis* were explained only by flower number (Table 2). In both species, individuals with more available open flowers received more pollinator visits. The probability that a *D. uncinatum* plant be visited by an insect was higher in low than high fire frequency ($Z = 2.719$, $P = 0.007$) and unburned scenarios ($Z = 2.549$, $P = 0.011$) (Online Resource 5). However, *D. uncinatum* individuals that were visited at least once (positive counts) received similar number of pollinator visits across all fire frequency scenarios ($Z < 1.644$, $P > 0.1$; Fig. 2).

Reproduction

Total flower production per individual of *D. uncinatum* was not affected by fire frequency, while flower production of *R. edulis* was marginally affected by fire frequency (Table 3a) showing the highest values in unburned condition (Fig. 3a, b). Moreover, the sampling period had significant effects on the variation of flower production in both plant species (Table 3a; Fig. 3a, b). The number of flowers produced in the second period was between 1.5 and 4 times higher than the first period across all sites for *D. uncinatum* and *R. edulis*, respectively (Fig. 3a, b). Fruit set variation in *D. uncinatum* was significantly explained by fire frequency (Table 3b). On average, individuals growing in unburned sites set around 2.8 times more fruits than individuals from both low and high fire frequency scenarios (unburned-low: $Z = 9.599$, $P < 0.001$; unburned-high: $Z = 10.195$, $P < 0.001$), which did not differ between them (low–high: $Z = 0.583$, $P = 0.829$; Fig. 3c). On the contrary, while there was a trend of decreased fruit set of *R. edulis* as fire frequency increased (Fig. 3d), it was not statistically

Table 1 Relative abundance (%) of floral visitors for two native Fabaceae species in Chaco Serrano sites subjected to different fire frequencies: unburned, low (1–2 fires) and high fire frequency (3–4 fires) in the 1991–2015 period

Pollinator species	Order	*Desmodium uncinatum*			*Rhynchosia edulis*		
		Unburned	Low frequency	High frequency	Unburned	Low frequency	High frequency
*Agraulis vanillae maculosa**	L	–	–	–	–	0.37	–
Apis mellifera	H	–	–	–	–	1.10	–
Bombus bellicosus	H	18.75	20.63	38.78	–	–	6.52
Bombus morio	H	15.63	–	–	–	–	–
Bombus opifex	H	31.25	19.05	44.90	4.24	–	8.70
*Chioides catillus**	L	–	1.06	–	–	–	–
Colletes sp.	H	–	0.53	–	1.69	–	–
Helophilus sp.*	D	–	–	2.04	–	0.74	6.52
Hesperiidae sp.*	L	–	–	–	–	0.37	–
Megachile sp.	H	28.13	17.46	14.29	6.78	18.38	17.39
Notanthidium sp.	H	–	–	–	13.56	5.51	8.70
Strymon sp.*	L	–	–	–	–	0.37	2.17
Trimeria sp.*	H	6.25	21.16	–	73.73	70.22	28.26
Xylocopa ordinaria	H	–	20.11	–	–	2.94	21.74

Data refer to the proportion of visits by particular species in relation to total number of visits per fire frequency scenario across two flowering periods (2013–2014 and 2014–2015). Order: diptera (D), hymenoptera (H), lepidoptera (L)

* Illegitimate floral visitor

Table 2 Effects of fire frequency (unburned, low and high fire frequency), flower number per plant and their interaction on pollinator visit number to *Desmodium uncinatum* and *Rhynchosia edulis*

Species	Effect	LRT	
		χ^2_1	*P* value
Desmodium uncinatum	Fire frequency	10.649	**0.031**
	Flower number	47.139	**<0.001**
	Flower number × fire frequency	5.921	0.205
Rhynchosia edulis	Fire frequency	5.429	0.246
	Flower number	45.627	**<0.001**
	Flower number × fire frequency	4.620	0.329

Significance of the effects were tested with likelihood-ratio test (LRT) of nested hurdle models

Values of $P < 0.05$ are shown in bold

different (Table 3b). Site as random factor showed a significant influence on fruit set variation in the two plant species (Table 3b). Period as random factor had no effects on fruit set variation in *D. uncinatum* but had a significant and higher influence than site on fruit set variation in *R. edulis* (Table 3b).

Relationships between fruit set and soil resources

In both species, the probability of setting fruits was positively related to the integrated soil quality variable (Fig. 4). For *D. uncinatum*, the relationship was much stronger than for *R. edulis* as observed from the steeper curve and the two-fold higher magnitude of the estimate. Because soil property variations were significantly explained by fire frequency (Online

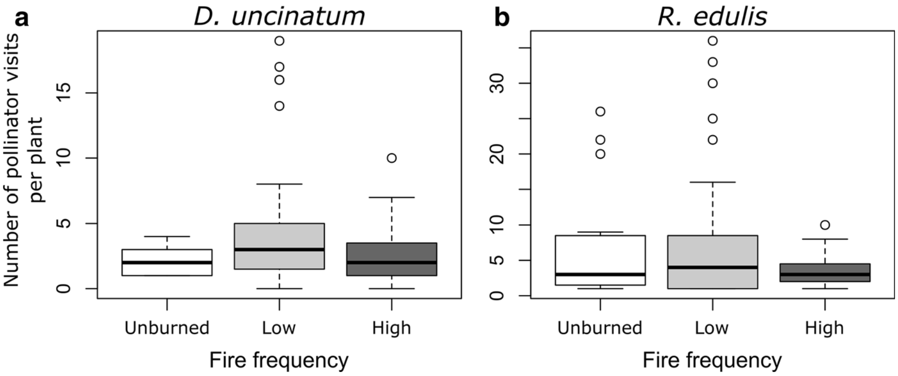

Fig. 2 Pollinator visits to *Desmodium uncinatum* (**a**) and *Rhynchosia edulis* (**b**) plants that received at least one visit during observation periods of 15 min per individual in Chaco Serrano sites subjected to different fire frequencies: unburned, low (1–2 fires) and high fire frequency (3–4 fires) in the 1991–2015 period. Data were pooled across two flowering periods (2013–2014 and 2014–2015)

Table 3 General linear mixed model on (a) flower production per plant and (b) fruit set (fruit/flower ratio) of *Desmodium uncinatum* and *Rhynchosia edulis*

Response variable	Species	Effect	Variance[a] (±SD)	LRT	
				χ^2_1	*P* value
(a) Flower production	*D. uncinatum* ($n = 193$)	Fixed			
		Fire frequency		2.036	0.361
		Random			
		Site	0.008 ± 0.087	3.087	0.079
		Period	0.144 ± 0.380	116.460	**<0.001**
	R. edulis ($n = 204$)	Fixed			
		Fire frequency		5.891	0.053
		Random			
		Site	0.022 ± 0.150	11.221	**<0.001**
		Period	0.359 ± 0.599	200.510	**<0.001**
(b) Fruit set	*D. uncinatum* ($n = 216$)	Fixed			
		Fire frequency		24.672	**<0.001**
		Random			
		Site	0.027 ± 0.165	83.079	**<0.001**
		Period	0.002 ± 0.044	2.451	0.117
	R. edulis ($n = 207$)	Fixed			
		Fire frequency		5.522	0.063
		Random			
		Site	0.042 ± 0.206	23.901	**<0.001**
		Period	0.096 ± 0.310	80.323	**<0.001**

Fire frequency is the fixed effect with three levels (unburned, low and high fire frequency), whereas site and period are random effects with nine and two levels, respectively. Significances of fixed and random effects were tested with a likelihood-ratio test (LRT)

Values of $P < 0.05$ are shown in bold

[a] Variance (±SD) indicates the absolute variation of each response variable due to random factors

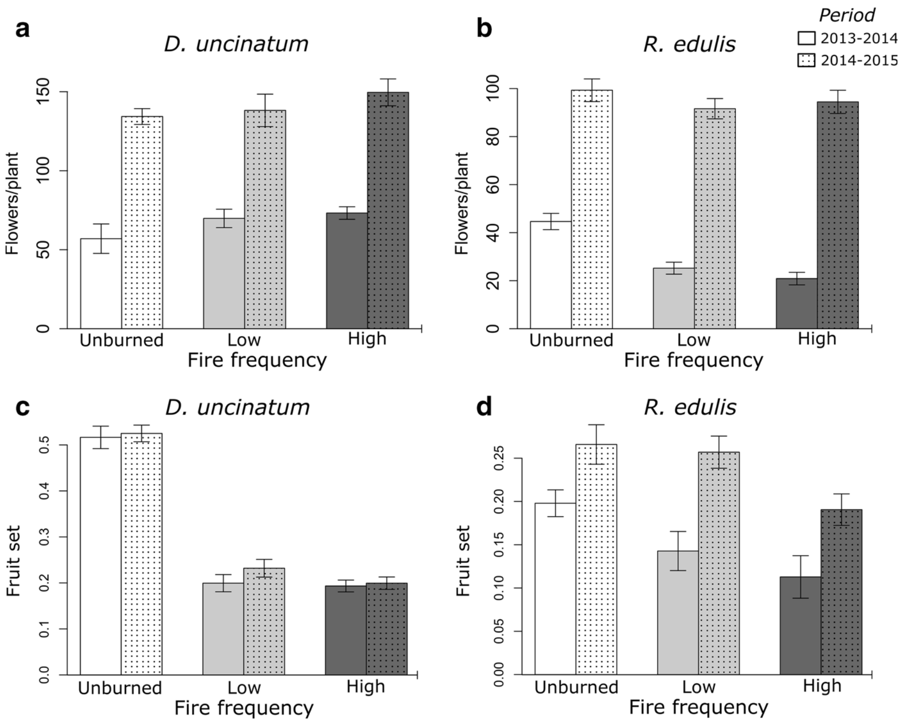

Fig. 3 Flower production per plant (**a**–**b**) and fruit set (fruit/flower ratio, **c**–**d**) of *Desmodium uncinatum* and *Rhynchosia edulis* in unburned, low fire frequency (1–2 fires) and high fire frequency sites (3–4 fires) in the 1991–2015 period, for two reproductive periods (2013–2014: *not dotted*, and 2014–2015: *dotted*). Values are mean ± SE

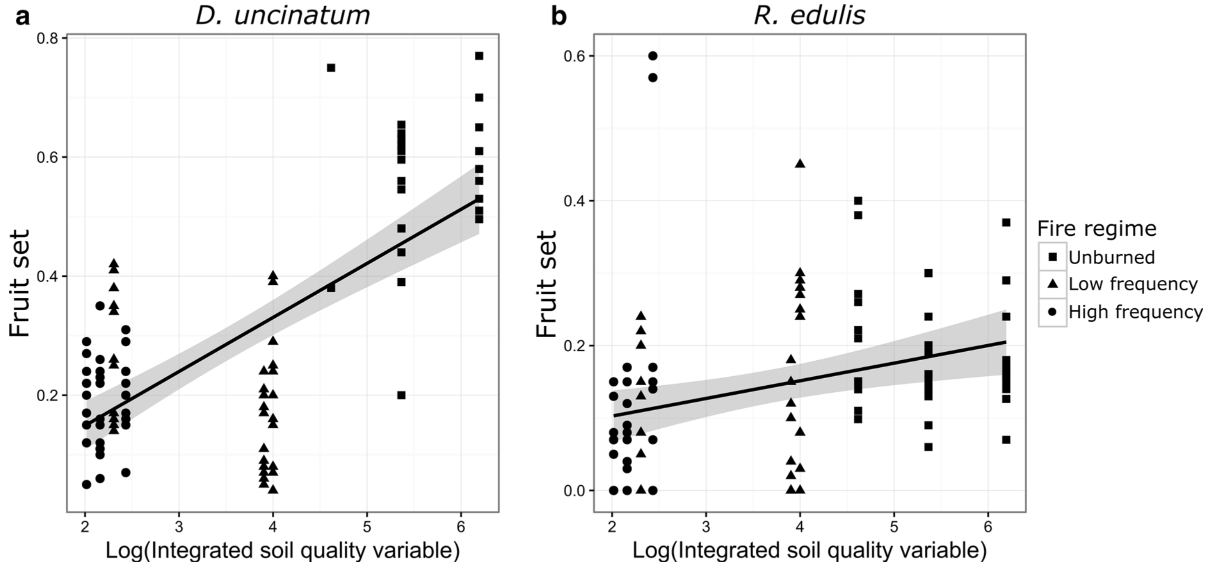

Fig. 4 Relationship between fruit set (fruit/flower ratio) of *Desmodium uncinatum* (**a**) and *Rhynchosia edulis* (**b**) during 2013–2014 period and the integrated soil quality variable (log-transformed) across different fire frequencies: unburned, low (1–2 fires) and high fire frequency (3–4 fires) in the 1991–2015 period. The relationship is significant for the two species (*D. uncinatum*: *Estimate* = 0.44; $Z = 24.20$; $P < 0.001$; *R. edulis*: estimate = 0.20; $Z = 5.54$; $P < 0.001$) according to GLM. *Dark shading* shows 95% confidence limits

Resource 2), and particularly water content, nitrates and electrical conductivity significantly decreased in burned sites (Fig. 1), fruit set in both plant species also followed the same pattern as these soil parameters, decreasing fruit set as soil quality decreased (Fig. 4).

Discussion

Our results partially support the initial stated hypotheses: while increased fire frequency eroded soil quality, it did not decrease floral abundance of the focal plants. As a result, richness, composition and visitation frequency of the pollinator assemblages visiting flowering individuals of the studied plant species were similar across the fire regimes, with the exception of increased visitation frequency to *D. uncinatum* in the low fire frequency sites. Nevertheless, the probability of setting fruits in the two legume species decreased in burned areas as a consequence of lowered resource availability in eroded soils triggered by frequent fires. Interestingly, soil resource limitation induced by fire frequency had stronger negative effects on fruit set of the exogamous *D. uncinatum* while the autogamous *R. edulis* showed a less drastic decrease in reproductive output.

Soils of low and high fire frequency sites showed a strong decrease in fundamental soil fertility parameters in relation to unburned sites, which are crucial for fruit maturation (e.g., Willson and Price 1980; Ågren and Willson 1992). Resource limitation for fruit production can result from insufficient soil nutrients, water or light availability, which increase abortion rates of flowers and/or young fruits and seeds (Willson and Price 1980). While nitrates and electrical conductivity appeared to respond to the effects of fire frequency, soil moisture showed a different pattern. Soil water content was highest in long-unburned sites but showed identically low levels in recently burnt sites of low and high fire frequency. Such result may suggest an effect of time-since-fire rather than fire frequency on water availability in recently burned sites. Both burned sites share the same time elapsed after the last fire event, which occurred in 2011, and thus they share microclimatic conditions of greater insolation, exposure to wind and litter cover, which drives a higher evapotranspiration and therefore a negative water balance for the plants (e.g., Pausas 2015). In this sense, both plant species clearly responded to increased water availability through precipitation as observed by the significant higher flower production across all sites in the wetter second season (2014–2015). Moreover, increased precipitation not only increased flower number but also fruit set in the autogamous *R. edulis.* Such positive response implies that water availability in response to time-since-fire plays an important role in flower production of both resprouting species, but that soil nutrient limitation induced by frequent fires also contributes to plan reproductive success.

Limitation of soil resources may not be the only mechanism through which frequent fire reduces reproduction in these two resprouting species. Repeated stem removal caused by too short fire intervals reduces the carbohydrates reserves that resprouting plants store in underground organs such as rhizomes and xylopodial roots (Bond and Midgley, 2001). Because resource allocation to storage implies a traded off cost against growth or reproduction (Obeso 2002; Bond and Midgley 2001), these two functions may decrease regardless of resource availability in the soil. While it has been demonstrated that the resprouting vigour is driven by the reserves stored in the plants (e.g., Bowen and Pate 1993; Canadell and Lopez-Soria 1998; but see Cruz et al. 2003), it still remains unexplained how sexual reproduction respond to plant storage reserve and nutrient availability of soils in fire-prone environments.

Richness and composition of pollinator assemblages visiting the two legume species were similar in all fire frequency scenarios across two consecutive years. At a local scale, the most immediate cause of such response pattern may be due to the similar floral abundance produced by both plant species across all fire frequency scenarios in both years. There was, however, natural variability in open available flowers among individuals within each site, and as expected, flower availability per plant was the only variable explaining pollinator visitation at the individual level (e.g., Ebeling et al. 2008), independently of fire frequency. Only a few previous studies have assessed fire frequency effects on pollinators, showing somewhat similar response patterns as found here. Lazarina et al. (2017) observed that bee richness was not affected by fire frequency but only altered bee composition, increasing heterogeneity of community between recurrently burned sites of a Mediterranean forest. Research in the past, however, has been largely oriented to assessing pollinator responses to a single fire event in comparison to unburned condition, focusing on post-fire effects (Ne’eman et al. 2000; Potts et al. 2001, 2003). Most studies have found decreased pollinator richness and abundance when measured shortly after the fire event (Ne’eman and Dafni 1999; Ne’eman et al. 2000). Interestingly, Moretti et al. (2006) observed that pollinator insects in temperate forests had medium resilience to a single fire, recovering 6–14 years after the fire event. However, when fire frequency increased the recovery time for pollinators was significantly delayed, increasing up to 25 years (Moretti et al. 2006). In our study system, both low and high fire frequency sites shared the same time elapsed after the last fire event in 2011. Thus, we may argue that pollination service to legumes native to Chaco Serrano communities is relatively resilient to the current frequency of one fire every five to 6 years, as these two dominant herbs provided sufficient floral resources for their insect pollinators to attain comparable pollinator/pollination levels to that of unburned communities.

A recent assessment of plant community in these same study sites shows that high fire frequency decreases plant richness and changes plant composition (Carbone et al. 2017), which may lower the quality and quantity of overall floral resources and potentially affect pollinator fauna community (Dafni et al. 2012; Brown and York 2016; Brown et al. 2016a). If pollinator fauna was in fact lowered in our frequently burned sites, one possible explanation for the similar or even higher pollination visitation observed to the studied plant species may be due to plant–plant competition for pollinators (Brown et al. 2016b). When pollinators and competing plant populations both decline, visitation to remaining plant species may not decrease because the scarce remaining pollinators focus their foraging on the remaining plant species (e.g., Potts et al. 2006). Under such circumstances, the plant species here studied would represent important resources that contribute to the conservation of native pollinators in burned environments. Furthermore, increased spatial heterogeneity shaped by different fire regimes (i.e., pyrodiversity) creates landscape mosaics of different successional stages that can provide habitat for multiple plant and insect species (Brown and York 2017; Kral et al. 2017). Pollinators can respond positively to heterogeneous landscapes because they may find feeding and nesting resources in surrounding environments (Brown et al. 2016b; Ponisio et al. 2016). Such heterogeneity may have also played a role in our observed similar pollinator assemblages in sites with different fire frequencies. Nevertheless, our study has the limitation of not being able to make interpretations beyond the population-level scale studied. Thus, further studies are needed to rule out or confirm such potential community and landscape effects of fire regime on pollinator assemblages.

Provided that individual plants of both species produced similar numbers of flowers and received a comparable pollinator visitation (or even higher in low frequency sites) across all fire frequency conditions, the reproductive success of plants in frequently burned sites depended mainly on the soil resources available for setting fruits. The magnitude of fruit set limitation by abiotic resources is likely to differ among plant species with different dependencies on animal pollination (e.g., Harder and Aizen 2010). Plants that rely entirely on animal pollinators for successful reproduction invest greatly in flower attraction, producing large, colourful and resource-consuming flowers with nectar and pollen as rewards (Strauss 1997; Harder and Aizen 2010; Schmid et al. 2015). Due to such high initial reproductive cost when growing in low resource environments, pollinator-dependent species saturate fruit production at a lower threshold of pollinator visitations as compared to growing in a high resource environment (Ågren and Willson 1992; Harder and Aizen 2010). In other words, the expected positive correlation between pollination visitation and reproductive success is no longer expected in such abiotic-depleted conditions, as they cannot afford to mature all fertilized flowers. As a result, plants of the exogamous pollinator-dependent *D. uncinatum* growing in low and high fire frequency scenarios aborted a larger number of expensive, larger and pluri-ovulate flowers or young fruits than plants growing in more resourceful unburned sites. Such abiotic resource dependency for setting fruits is evidenced by its highly positive relationship with soil fertility. A similar response pattern was observed in pollinator-dependent herbs in fire-prone landscapes that had reduced reproductive output due to abiotic resource limitation, particularly water, but pollinator visitation was not affected (Brown and York 2016). In contrast, because the autogamous *R. edulis* can set fruits in the absence of pollinators and it produces inexpensive small and bi-ovulate flowers with scarce levels of nectar, this species was able to cope better in terms of setting fruits, being comparatively less affected by lower resource availability in both low and high fire frequency sites. While *R. edulis* showed a non-significant trend of lower fruit set as fire frequency increased, it showed a weaker but significant positive relationship with soil quality variables.

The population viability of resprouting species depends on the regeneration of individual plants more than seedling recruitment (Hoffmann 1998). However, because species with this persistence strategy possess a limited potential for clonality (Clarke et al. 2013), their unique long-term population viability strategy relies on the possibility of generating sexually viable progeny through seeds. The amount of progeny produced in a reproductive event defines the maximum population recruitment potential for the next generation (Wilcock and Neiland 2002). Thus, decreased reproductive output, as observed here in both species in frequently burned sites across two consecutive years, it is likely to reduce seedling recruitment, seriously affecting their long-term viability. In addition to reduced reproductive output, we previously observed that these same populations of both species growing in frequently burned sites had lowered specific leaf area (Carbone and Aguilar 2016), which imply a reduction of individual plant growth rates. Thus, fire can affect demographic processes such as growth, reproduction and mortality of plant species (Carbone and Aguilar 2016; Tulloch et al. 2016), and potentially affect the pollinator populations that depend on these native plants. This study contributes to disentangling the ecological mechanisms through which fire shapes plant reproduction in Chaco Serrano environments, which involves the interactions between soil, plants and pollinators. We highlight the need for integrating different fire regime factors (time-since-fire and frequency) at local and landscape scales to allow performing better recommendations on the management and conservation of these natural resources in fire-prone ecosystems.

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