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Abiotic and biotic interactions as drivers of plant reproduction in response to fire frequency

Lucas M. Carbone[1,2] · Ramiro Aguilar[1,3]

Abstract

Plant reproduction is influenced by abiotic resources and biotic mutualistic and antagonistic interactions, which in turn can be affected by anthropogenic disturbances such as increased fire frequency. Because frequent fires deplete soil fertility and thus decrease resource availability for plants, we hypothesize that increased fire frequency decreases specific leaf area (SLA) and reproductive success. In addition, lower SLA levels in frequently burned sites should decrease herbivore damage because of reduced leaf palatability. Finally, increased fire frequency will have stronger negative effects on specialist insects (seed predators) as compared to generalist feeding insects such as herbivores and pollinators, which can have direct consequences on plant reproduction. Through an integrative path analytical approach, we assess fire frequency effects on the reproductive success of two resprouting legumes from the Chaco Serrano (*Desmodium uncinatum* and *Rhynchosia edulis*), estimating the relative importance of SLA along with pollination, insect herbivory and seed predation interactions. Increased fire frequency decreased SLA but it did not affect biotic interactions in both plant species, with the exception of increased leaf herbivory in *R. edulis*. Sexual reproduction of *D. uncinatum* was reduced in burned sites but it remained similar across burned and unburned sites in *R. edulis.* Within burned areas, both species efficiently maximized the allocation to reproduction, showing a conservative strategy in the use of resources when SLA levels are extremely low. Decreased plant fecundity, especially in *D. uncinatum*, is likely to impact on the density and long-term viability of populations growing in anthropogenic high fire frequency areas.

Keyword Chaco serrano · Herbivory · Path analysis · Pollination · Seed predation · Specific leaf area

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Introduction

Plant reproductive success is influenced by several factors, including abiotic resources and biotic mutualistic and antagonistic interactions, which in turn can be affected by habitat disturbances (Maschinski and Whitham 1989; Burd 1994; Ashman et al. 2004; Aguilar et al. 2006, 2019). Fire is one of the most important disturbances across the Earth that modulates the ecological and evolutionary dynamics of plants and animals of many ecosystems (Bond et al. 2005; Pausas and Keeley 2009; Pausas 2019). Currently, anthropic activities have altered natural fire regimes, increasing their frequency and thereby affecting multiple ecological and evolutionary processes in plant and animal populations (Koltz et al. 2018; Keeley and Pausas 2019). Two major effects of increased fire frequency involve changes in soil properties and biotic interactions. High fire frequencies can affect soil fertility, decreasing organic matter and nitrogen and also alter microenvironmental variables of soil surface

(Pellegrini et al. 2015, 2018), all of which may affect vegetative and reproductive traits of plants (Reich et al. 1990; Rieske 2002; Kilkenny and Galloway 2008; Kowaljow et al. 2018). In addition, frequent fires often reduce the diversity and/or change the composition of animal pollinators as well as herbivores and seed predators (Winfree et al. 2009; Kral et al. 2017; Carbone et al. 2019; Simanonok and Burkle 2019). Such changes in both mutualistic and antagonistic interactions can have direct consequences on plant reproductive success.

Resource limitations induced by increased fire frequency can drive intraspecific changes in main vegetative functional traits (Albert et al. 2010; Dantas et al. 2013; Rosbakh et al. 2015). Specific leaf area (SLA hereafter) is a key functional trait indicative of plant growth rate, photosynthetic rate, and the type of resource-use strategies, from acquisitive to conservative (Díaz et al. 1998; Wright et al. 2004; Anacker et al. 2011; Pérez-Harguindeguy et al. 2013). In particular, soil resource limitation in frequently burned sites may decrease SLA at the intraspecific level, which is associated to lower photosynthetic and growth rates and consequently to less resources allocated to fruit and seed set (Carbone and Aguilar 2016, 2017). Furthermore, a reduction in SLA induced by recurrent fires may decrease the damage levels by herbivores due to lower leaf palatability and higher physical defenses (Adams and Rieske 2003; Wright et al. 2004; Augustine and Milchunas 2009), potentially allowing more resources to reproduction. Therefore, changes in SLA might influence plant reproductive performance through abiotic (resources) and biotic (herbivory) mechanisms in fire-disturbed habitats.

Animal pollination is a selective force for the vast majority of angiosperms, as it affects the quantity and quality of the offspring produced in a reproductive event (Burd 1994; Wilcock and Neiland 2002). However, reproductive success is likely to present ecological compromises in response to other opposite selective forces (Brody 1997). For example, herbivory decreases photosynthetic leaf area, which can limit resource availability for flower and seed production, which may deplete sexual plant reproduction (e.g. Haas and Lortie 2020). In addition, pre-dispersal seed predation is an important selective pressure that determines the amount and quality of the surviving progeny, affecting the recruiting potential of plant populations (Crawley 2000). Because of the tight relationship between these biotic interactions and plant reproductive success, any alteration in richness or abundance of animal interacting partners due to increased fire frequency should have large effects on plant population demography.

While fire may reduce animal populations by the direct effect of flames, it can also boost richness and abundance of several species by increasing the quality and/or quantity of available food resources (Swengel 2001; Andersen 2003; Pausas 2019). For example, insect pollinators and some herbivores can increase in richness and abundance immediately after a fire event (Swengel 2001; Moretti et al. 2006; Winfree et al. 2009; Brown and York 2017; Carbone et al. 2019). Such an increase is mostly due to recolonization of individuals from neighboring unburned areas triggered by the increased food and nesting resources and reduced competition, which usually takes place after fire (Pausas 2019; Carbone et al. 2019). Highly mobile and generalist-feeding insects such as bees and grasshoppers, are often benefited during early post-fire succession (Whelan 1995; Swengel 2001; Kral et al. 2017; Peralta et al. 2017). However, factors of the fire regime such as time since the last fire and especially the frequency and severity of fires can negatively affect the insect response (Carbone et al. 2019; Lazarina et al. 2019; Simanonok and Burkle 2019). In sites with abiotic resources limitation induced by recurrent fires, the higher development of physical defenses against herbivores due to lower growth rates and changes in C/nutrients ratio in the leaves may actually reduce the attack from folivorous insects (Boege and Dirzo 2004). Seed predators represent another important antagonist insect group, among which bruchids (Coleoptera) represent the main group affecting progeny survival among Fabaceae species (Center and Johnson 1974; Janzen 1980). Pre-dispersal seed predation is often a more specialized antagonistic interaction (Huignard et al. 1990), which is highly species-specific in the Fabaceae from tropic, subtropic, and arid environments worldwide (Janzen 1980; Huignard et al. 1990; Kingsolver 2004). Highly specialized interacting insect species, either mutualist or antagonist, can be more susceptible to fire effects than generalist ones because they are tightly dependent on specific host plants and habitat characteristics (García et al. 2016, 2017; Peralta et al. 2017; Koltz et al. 2018). Thus, reduced specialist seed predator abundance after fire should improve the survival success of seeds generated in a reproductive event.

In this study, we evaluate the effects of anthropogenically increased fire frequency on the reproductive success of two native resprouting herbs of the Chaco Serrano, *Desmodium uncinatum* and *Rhynchosia edulis* (Fabaceae). By means of a path-analytic approach, we simultaneously assess for the first time the relative importance of a key vegetative trait (SLA) and of the mutualistic (pollination) and antagonistic (herbivory and seed predation) interactions they display in contrasting fire frequency scenarios. Because high fire frequency in the Chaco Serrano depletes soil resources (Carbone & Aguilar 2016; Kowaljob et al. 2018; Giorgis et al. unpubl.), we expect to find decrease SLA in both species (Fig. 1). Moreover, depleted soil fertility also implies less resource availability to assign to reproduction. Similarly, lower SLA levels should decrease herbivore damage because of reduced palatability of the leaf tissue. Finally, increased fire frequency should reduce more drastically specialist interacting species such as seed predators, in comparison to

Fig. 1 Hypothesized relationships determining the reproductive success of *Desmodium uncinatum* and *Rhynchosia edulis*. Fire frequency may have direct and indirect effects on reproductive success through specific leaf area (SLA) and biotic interactions such as herbivory, pollination (pollinator visitation frequency) and pre-dispersal seed predation. Seed predation is not quantitatively represented in *Desmodium*. Solid and dashed arrows indicate positive and negative effects, respectively
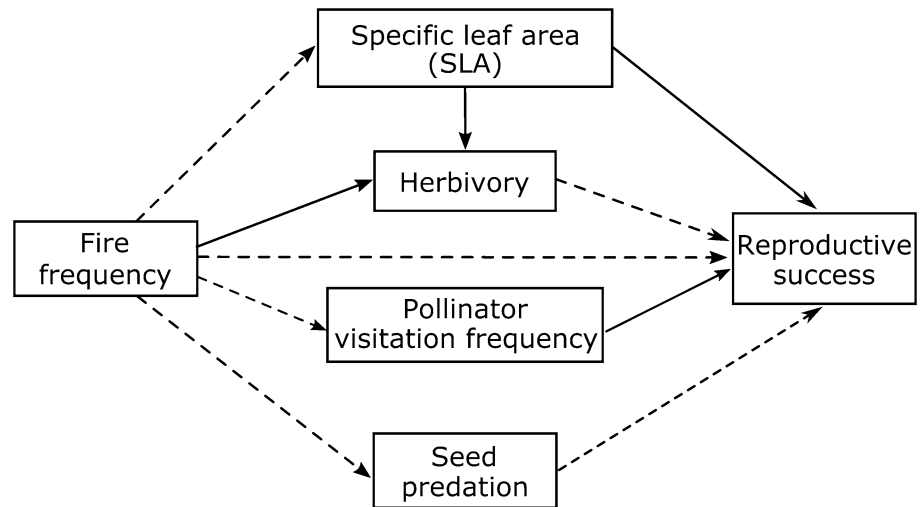
the more generalist leaf herbivore and pollinator species. In synthesis, by measuring the relationships between abiotic and biotic factors we expect to unravel the potential mechanisms affecting the reproductive success of two common herbs in Chaco Serrano ecosystems subjected to anthropogenically increased fire frequency.

Materials and methods

Study species

Desmodium uncinatum (Jacq.) DC. and *Rhynchosia edulis* Griseb. (hereafter *Desmodium* and *Rhynchosia*) are perennial herbs (Online Resource 1 and 2), widely distributed in subtropical mountain ecosystems from the United States to central Argentina. These plants are common herbaceous species from the Chaco Serrano ecoregion (Giorgis 2011), especially present in fire-prone environments (Carbone and Aguilar 2016). These species have woody rhizomes and xylopodium, which allow them to survive frequent fires and to regenerate by underground resprouting few days after fire (i.e. obligate resprouters, Online Resource 1b and 2b). Individuals of both species flower early and set fruits within the growing season following the fire event (Carbone and Aguilar 2016). Both species have low vegetative multiplication ability; therefore, sexual reproduction is the main strategy for long-term population viability (Carbone 2017).

Both species have typical papilionate flowers and are mainly pollinated by bees, which visit their flowers searching for both nectar and pollen (*Rhynchosia*) or only pollen (*Desmodium*). Both species are self-compatible but with key differences in their reproductive biology: *Desmodium* is mainly outcrossing, with an essential dependence on generalist social bumblebees (i.e., the absence of bumblebees decreases reproductive success by > 70%; Klein et al. 2007;

Alemán et al. 2014); *Rhynchosia* is mostly autogamous having no dependence on the solitary bees that visit its flowers (i.e., reproductive success is similar in the presence or absence of pollinators; Klein et al. 2007; Figueroa-Fleming 2014; Carbone and Aguilar 2017).

Both species experience leaf herbivory by generalist grasshoppers (Orthoptera), which can produce great damage to the leaves and reduce considerably their photosynthetic area. In *Rhynchosia*, adult bruchids (Coleoptera, Bruchidae) deposit eggs next to the ovary during flowering and the larvae develop fully inside the seeds, feeding on their reserves. The few information available on this interaction indicates that seed predation is conducted by a single bruchid species, thus its life cycle depends entirely on this host plant. Pre-dispersal seed predation in *Desmodium* is totally unknown (Carbone 2017).

Studied sites and sampling

The study was conducted in the eastern hillsides of Sierras Chicas from Córdoba, Argentina. The vegetation consists of subtropical dry forest intermingled with shrublands and grasslands conforming a composite mosaic of physiognomies, which can vary in plant composition. This complex landscape configuration is determined by the impact of disturbances such as fire and livestock grazing pressures (Luti et al. 1979; Gavier and Bucher 2004). The Sierras Chicas is the mountain system of central Argentina most affected by fire in total area and frequency, with 297.125 ha out of 812.663 ha burned between 1999 and 2019, which is equivalent to 36.6% of its area, and with sites that register up to 5 or 6 fires in just 17 years (Argañaraz et al. 2015; Argañaraz 2016). We compiled the fire history from 1991 to 2015 period (24 years) based on different databases covering approximately a 40 km^2 focal area (31° 05′ 38.53″ S to 31° 09′ 11.73″ S and 64° 24′ 10.49″ W to 64° 20′ 40.35″

W). We used fire records registered by Civil Defense of Rio Ceballos city (Giorgis et al. 2013) and Landsat TN and ETM satellite images (Argañaraz et al. 2015). We selected nine sites with different fire regimes: six burned sites along a gradient of fire frequency (from one to four fire events) and three unburned sites (see Carbone and Aguilar 2016 for site specifications). All the burned sites shared the same time elapsed since the last fire event, which occurred in 2011 (i.e. samplings were performed 3 years after the last fire) and all of them were subjected to similar low to moderate fire intensity. All sites were selected with the criteria of comparable altitudinal position (820–1200 m asl), slope exposure (N) and topographic position (middle slope). Sampled sites are located in private properties with similarly low stocking rates (cattle load) and separated by a minimum distance of 500 m from each other. The unburned sites were represented by a vegetation physiognomy of open native forest with higher vertical structure and a larger tree layer than the burned sites, which showed a shrubland structure dominated by a higher cover of shrubs and herbs (Carbone et al. 2017). Spatial distribution and specification of studied sites can be seen in Carbone and Aguilar (2016).

In each of the nine sites, we marked 12 adult individuals of *Desmodium* and *Rhynchosia* at their reproductive stage. On each of these individuals we simultaneously measured SLA, pollination, leaf herbivory and seed predation levels, along with female reproductive success. The sampling was conducted during the warm season from December 2014 to March 2015, 3 years after the last fire (2011) for most of the burned sites. As an indicator of relative growth rate and physical feature of the leaves (hardness and palatability) we measured the specific leaf area (SLA) functional trait. For this, we randomly selected five fully expanded green leaves of similar age, discarding the base and tip leaves of the branches, concurrently in all sites. We calculated SLA (cm^2/g) by dividing leaf area (calculated by scanning the fresh leaves and then using ImageJ 1.47v software) and leaf dry mass (measured with a precision digital scale), according to standardized protocols (Pérez-Harguindeguy et al. 2013).

To quantify plant–pollinator interaction, we recorded all floral visitors in periods of 15-min observations per individual plant across the entire flowering period in six individual plants per site. Observations were conducted by direct focal observation and through HD video cameras at moments of maximum floral display (i.e., when most of the flowers per inflorescence were open), which occurred from 8:00 to 12:00 h for *Desmodium* and from 12:00 to 16:00 h for *Rhynchosia*. The overall sampling effort across the flowering period was similar among sites, totaling ca. 20 h of observations for each plant species. In each observation, we registered the number of open flowers, the number of visited flowers and the taxonomic identity of each

floral visitor. We only considered legitimate pollinations, which implied that pollinators effectively contacted the fertile floral whorls. We defined the frequency of legitimate pollinator visits as the number of visits/number of available flowers/time of observation period. This form of calculation allows controlling for the effect of flower offer per plant, standardized by the proportion of visited flowers per minute.

Natural levels of herbivory were estimated as the percentage of leaf area consumed by chewing insects in five randomly selected leaves per plant on the 12 plants per site. We used six visual damage categories corresponding to a specific range of consumed leaf area: 0 = 0%, 1 = 0–6%, 2 = 6–12%, 3 = 12–25%, 4 = 25–50%, 5 = 50–100% (Dirzo and Domínguez 1995). These categories were used because low levels of damage were the most frequent and thus requiring narrower ranges of damage per category, while high levels of damage were rare, and therefore, they can be grouped into broader range intervals (Boege and Dirzo 2004). Then, we calculated the herbivory index (*HI*) per individual plant: $HI = (\sum C_i * n_i)/N$, where C_i corresponds to the category of damage, n_i is the number of leaves in the i_{th} category of damage, and N is the total number of leaves assessed per plant (Dirzo and Domínguez 1995).

To assess pre-dispersal seed predation levels, we collected all mature fruits produced by the selected individual plants of *Desmodium* and *Rhynchosia* at the end of the reproductive season. Fruits were stored at room temperature and were monitored for at least 45 days until fully development of larvae and adult emergence. Adult predators were collected and conditioned for later identification. Predated seeds were identified by observation of the circular hole left by the adult predator after emergence (Online Resource 2). Seed predation was calculated as the percentage of predated seeds in relation to total seed production per maternal plant. Because emerging bruchid adults did not re-infest the seeds, predation of stored seeds did not continue in lab conditions. All predated seeds were completely viable and showed higher germination in relation to healthy seeds because bruchids break the physical dormancy of the *Rhynchosia* seeds without damaging its embryo (Martinat 2012; Carbone 2017). However, seedling development from predated seeds showed higher mortality than non-predated seeds due to high levels of post-germination fungal infection (Carbone 2017).

To estimate plant reproductive success, we marked at least six inflorescences and counted the number of flowers and later the fruits produced by natural pollination from each marked inflorescence in both plant species (12 plants per site). Fruit-set was calculated as the number of mature fruits/ number of marked flowers. We also collected all fruits from each marked inflorescence and counted the number of seeds and unfertilized ovules per fruit and calculated seed-set as number of healthy seeds per fruit/mean number of ovules

per flower. Female reproductive success was calculated by multiplying fruit-set and seed-set.

Data analysis

We used confirmatory path analysis to assess the effects of fire frequency on different variables that can affect plant reproductive success. It allows obtaining the direction and magnitude of each of the direct effects (path coefficients) on the response variable (Mitchell 2001). The main purpose of path analysis is confirming an agreement between specific causal hypotheses and empirical data, which is assessed through a goodness-of-fit statistic between the observed and expected correlations (Mitchell 2001; Shipley 2013). We established a causal relationship model to assess whether fire frequency, (i.e., the independent variable), measured as the number of wildfires of each of the nine sites (from none to four fire events in the last 24 years), affects the reproductive success of *Desmodium* and *Rhynchosia* plants through SLA and biotic interactions such as pollination, herbivory and seed predation (i.e., dependent variables). Path analysis was performed with standardized variables and path coefficients were obtained through partial regression coefficients of the relationship between the independent variable (fire frequency) and a dependent variable (e.g. herbivory) at a time, with statistical control (all else statistically held constant). Path coefficients indicate the degree of expected variation in a dependent variable to changes in one unit of the independent variable, expressed in standard deviation values. Based on the general hypothetical model (Fig. 1), we obtained more parsimonious nested models by removing non-significant path coefficients. The goodness-of-fit of the models and the method of selection were based on the Akaike's information criterion (AIC) using tests of directed separation, which evaluate the assumption that the specific causal structure reflects the data (Shipley 2013). This method involves a correction for small sample sizes and implies an advantage of the d-sep test over the classical structural equations models as one can use different functional forms for the links between the variables and for the distributional assumptions of the random components (Shipley 2013; Lefcheck 2016). This approach is appropriate for our data set with relatively low sample units per site and variables with non-normal error distributions. All analyses were performed in R (R Core Team 2020) using general and generalized linear mixed-effect models (*lme* and *glmer* function from the *nlme* and *lme4* package, respectively) with the site identity as a random factor to control for the intrinsic hierarchy product of the experimental design. The d-separation tests, path coefficients and the goodness-of-fit for each model were calculated with the *psem* function from the *piecewiseSEM* package (Lefcheck et al. 2019). We estimated the goodness-of-fit based on Fisher's C statistic, which is

a maximum-likelihood estimate (Shipley 2013). For the fit of models, we evaluated the differences between observed and expected correlations, with the null hypothesis that the data fit the implicit covariation structure in the model. Their acceptance ($P > 0.05$) indicates a good fit of the data under the proposed model, while the model is rejected if the P value is lower than the chosen significance level, $\alpha = 0.05$ (Shipley 2013). The C value associated with each model was used for calculating the AIC with correction for small sample sizes: $AIC_c = C + 2\,K\,[n/(n - K - 1)]$, where K is the number of parameters estimated by each model and n is the sample size. The model with the lowest AIC_c is the one that presents the best fit. The comparison among models was calculated by ΔAIC_c relative to the best-fitting model of the set. The scripts of analyses and the datasets used are provided in Electronic Supplementary Material (Online Resource 3–5). We performed the analyses with two datasets: one including individuals with some non-available data (NAs; mostly pollination interactions) and one excluding all these individuals with NAs entries, finding very similar response patterns.

Finally, we used linear mixed-effect models (*lmer* function from the *lme4* package) to test the effect of SLA and fire condition, *i.e.*, unburned vs burned (all fire frequency sites), on the reproductive success of the two plant species. Site identity was used as random factor. After checking assumptions and fit of models by REML, significance of fixed effects was assessed with t tests (Satterthwaite's method).

Results

Biotic interactions

Frequency of pollinator visits to *Desmodium* and *Rhynchosia* flowers was, on average, 0.02 flowers per minute and very similar across sites with different fire frequency (burned and unburned); i.e., only 3% of the open flowers per plant were visited. The few pollinator visits to *Desmodium* were mainly led by *Bombus* spp., while in *Rhynchosia* most visitations were represented by *Megachile* sp. and *Nothantidium* sp. (Megachilidae) solitary bees and to a lesser extent by carpenter bees (*Xylocopa atamisquensis,* Online Resource 1 and 2; see Carbone and Aguilar 2017).

Herbivore damage to leaves of the two legumes was mostly caused by chewing insects, mainly nymphal and adult stages of grasshoppers (Orthoptera, Acrididae; Online Resource 1 and 2) and to a lesser extent by Lepidoptera larva. The leaf area consumed by these phytophagous insects on *Desmodium* individuals was 3.2% ($HI = 1.06$, SD ± 0.57) in unburned sites and 3.8% ($HI = 1.28 \pm 0.52$) in highest fire frequency sites (3–4 fires). In *Rhynchosia*, we observed herbivory levels of 1.8% ($HI = 0.63 \pm 0.39$) in unburned sites

but much higher herbivory levels of 4.3% ($HI = 1.48 \pm 0.58$) in sites with the highest fire frequency.

Pre-dispersal seed predation in *Rhynchosia* was only represented by one bruchid species, *Acanthoscelides* sp. (Coleoptera, Bruchidae; Online Resource 2), which presented a maximum number of one bruchid larvae per seed. For this plant species, bruchid infestation levels were slightly higher in unburned sites ($18\% \pm 0.20$) compared to high fire frequency sites, which showed an average predation level of $12\% \pm 0.15$. We recorded only two bruchid individuals in *Desmodium* seeds across the nine studied sites, corresponding to one species of *Meibomeus* (Bruchidae; Online Resource 1). Due to the extremely low abundance of this interaction, *D. uncinatum* could be a secondary host of the bruchid found, and therefore we discarded this interaction from the analysis.

Path analysis

The proposed causal relationship model to assess the effects of fire frequency on the reproductive success of *Desmodium* and *Rhynchosia* mediated by SLA and biotic interactions (pollination, herbivory, and seed predation) significantly explained the observed variation of the data (model 1, Table 1). However, the simpler alternative nested model (model 3, Table 1) showed a better fit than the initial model according to ΔAIC_c in both species. Both of them indicated a significantly strong negative effect of fire frequency on SLA (Fig. 2). The reproductive success of *Desmodium* was positively related to pollinator visitations, but it also showed a slight decrease with fire frequency (Fig. 2a). With the exception of a positive effect of fire frequency on insect herbivory in *Rhynchosia* leaves (Fig. 2b), increased fire frequency had no effect on any of the other biotic interactions. In turn, while SLA had a positive effect on reproductive success of *Rhynchosia*, the higher herbivory levels induced by fire frequency had no significant influence on its reproductive output (Fig. 2b). In addition, variations in SLA induced

by fire frequency did not affect herbivory levels on any of the two species.

Female reproductive success showed different relationships with SLA depending on the fire conditions (Fig. 3). In burned sites, regardless of the frequency, both species showed a negative relationship between SLA and reproductive output (more evident for *Desmodium*), i.e. as SLA increases, the reproductive success of plants growing in burned sites decreases. Interestingly, in both species there is a remarkable shrinkage in SLA range values in burned conditions, which is constrained to less than 300 cm^2/g (Fig. 3). Noticeable, the scale of SLA values in individuals growing in unburned sites is almost twofold larger in both species. Reproductive success of *Desmodium* individuals growing in unburned sites showed no relationship with SLA, and it was nearly twice as large as the reproductive success of individuals growing in burned sites ($t = 7.646$, $p < 0.0001$, $N = 82$; Fig. 3a). In contrast, *Rhynchosia* individuals in unburned sites showed a positive relationship between SLA and reproductive success (Fig. 3b), but range values of reproductive success were similar among individuals growing in burned and unburned conditions ($t = 0.269$, $p = 0.793$, $N = 93$; Fig. 3b).

Discussion

Our results show that increased fire frequency decreases specific leaf area (SLA), a key resource-acquisition trait, and does not affect biotic interactions in two resprouting herbs from the Chaco Serrano, with the exception of increased leaf herbivory levels in *Rhynchosia*. As a result, sexual reproduction is reduced in burned sites in *Desmodium* but it remains mostly stable across burned and unburned sites in *Rhynchosia*. Higher sunlight availability along with lower soil moisture and nutrients in abiotically stressed, frequently-burned sites can reduce photosynthesis, relative growth rate, and plant biomass (Violle et al. 2007; de Souza et al. 2016),

Table 1 Model fit of three competing path models determining the reproductive success of *Desmodium uncinatum* and *Rhynchosia edulis*

Species	Structural Equation Model	C	df	P	AIC$_c$	ΔAIC$_c$
Rhynchosia	3. SLA~FF, H~FF+SLA, RS~SLA+H+SP+FF	2.78	4	0.595	53.26	0.00
	2. SLA~FF, H~FF+SLA, RS~SLA+H+PVF+SP+FF	5.27	8	0.727	62.09	8.83
	1. SLA~FF, H~FF+SLA, PVF~FF, SP~FF, RS~SLA+H+PVF+SP+FF	13.63	10	0.190	125.83	72.57
Desmodium	3. SLA~FF, H~FF, RS~SLA+PVF+FF	6.08	8	0.638	43.55	0.00
	2. SLA~FF, H~FF+SLA, RS~SLA+H+PVF+FF	1.26	4	0.868	44.13	0.58
	1. SLA~FF, H~FF+SLA, PVF~FF, RS~SLA+H+PVF+FF	1.15	4	0.886	59.15	15.6

Fisher's *C* is the statistic used for global goodness-of-fit in the tests of directed separation, *df* is the degrees of freedom and *P* the null probability, AIC$_c$ is the Akaike value corrected for small sample sizes and ΔAICc is the difference in relation to the best-fitted model (model 3).
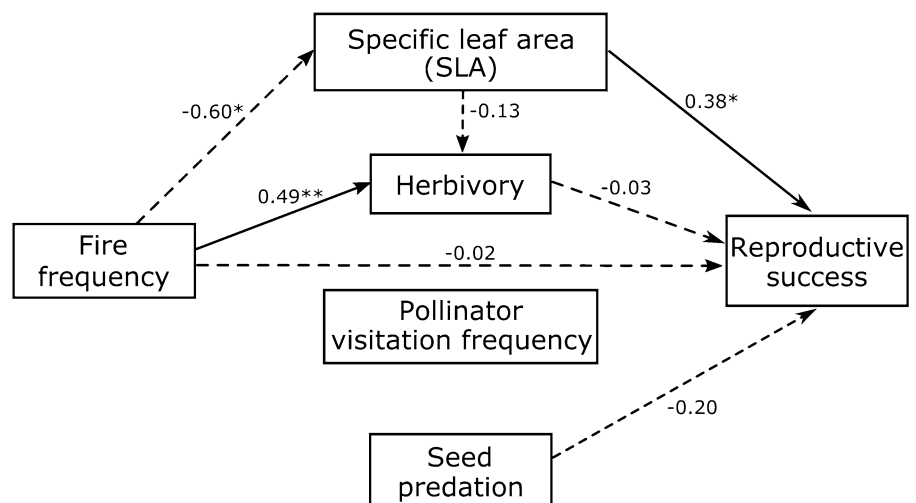
SLA specific leaf area, *FF* fire frequency, *H* herbivory, *RS* reproductive success, *SP* seed predation, *PVF* pollinator visitation frequency

A *Desmodium*
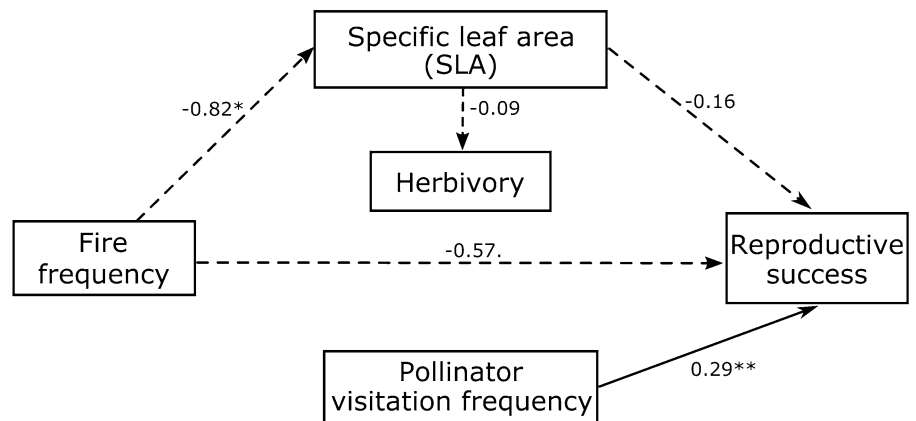
B *Rhynchosia*

all of which is translated into a reduction in SLA of perennial plants (Huang and Boerner 2008; Carbone and Aguilar 2016; de Souza et al. 2016).

From an ecophysiological view, lower SLA shaped by high fire frequency represents a plastic response indicative of a more conservative resource use strategy (Carbone 2017). However, individuals of both plant species growing in burned sites showed significant negative relationships between SLA and reproductive success: the lower the SLA the higher reproductive output. Such negative relationships indicate that despite the overall more conservative foliar strategy in burned sites, there is still enough variability in SLA to allow improvement in reproductive investment (Wright et al. 2004; Bricca et al. 2020). Interestingly, *Rhynchosia* plants growing in unburned sites showed a completely opposite strategy: individuals with higher SLA

(i.e. a more acquisitive strategy) showed higher reproductive success. This suggests that resprouting plants species with large underground storage organs such as *Rhynchosia,* can express a double strategy of resource use in response to fire: conservative but variable enough to have individuals with low SLA and high reproductive success in burned habitats, and acquisitive in unburned areas where there is no resource limitation and the reproductive success of individuals increase as the SLA increases.

The development of underground storage organs represents an advantageous strategy for the persistence of most of the perennial herbs and shrubs from Chaco Serrano that lose their aerial biomass and resprout after fire events (Fuentes et al. 2011; Carbone 2017; Schafer and Mack 2018). The available resources are likely to be mobilized from underground storage organs to reproductive structures (Schafer

Fig. 3 Relationship between the specific leaf area (SLA) and female reproductive success of *Desmodium uncinatum* (**a**) and *Rhynchosia edulis* (**b**) in burned (black) and unburned (grey) conditions

A *Desmodium*
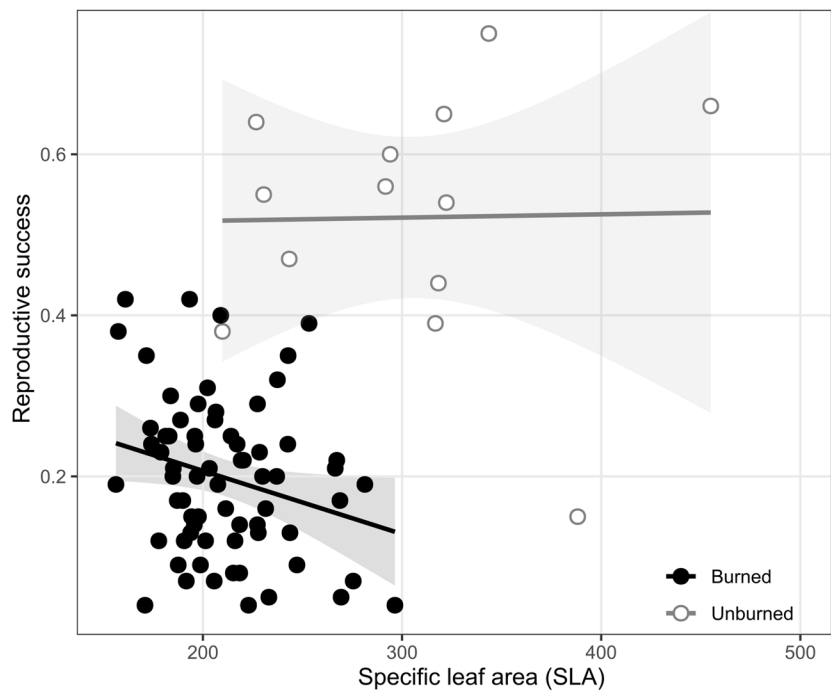
B *Rhynchosia*
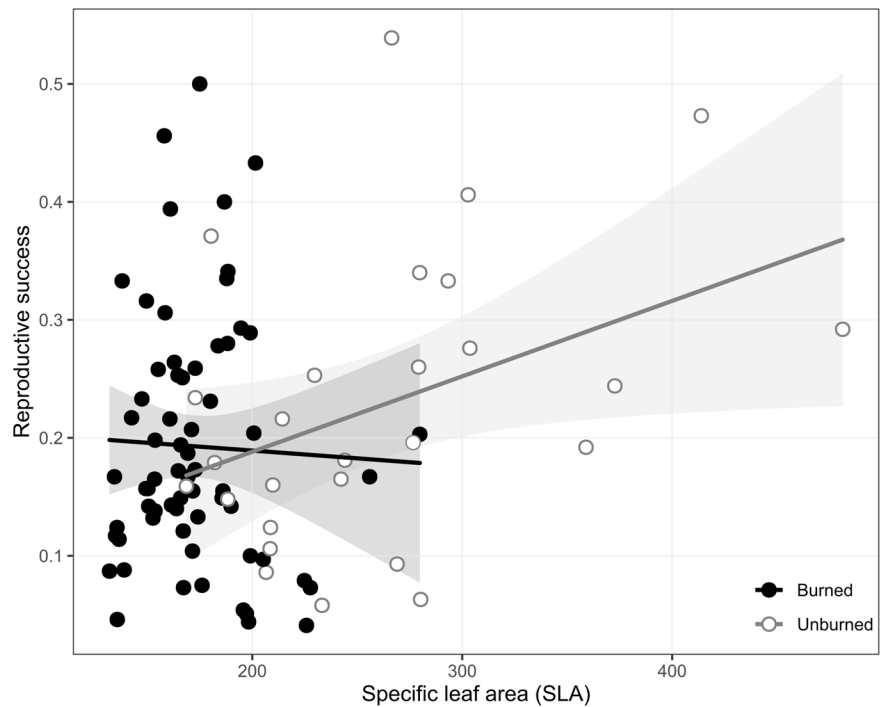
and Mack 2018). However, there are still no studies evaluating whether species with different underground organs can differentially modulate the resource storage and allocation to reproduction in response to fire in Chaco environments. Our results suggest that plant species with large underground storage organs, like xylopodium in *Rhynchosia* (Online Resource 2), may be more tolerant to resource limitation due to recurrent fires by redirecting their resources to reproduction than plants whose storage organs have less reserve capability (like rhizomes in *Desmodium,* Online Resource

1). By assessing intraspecific trait variability in different plant organs, we may learn whether there are specific causal relationships or adaptive responses in sites where the natural fire regime has been anthropogenically altered. Our results indicate that the current high fire frequencies in Chaco Serrano represents an ecological, and potentially evolutionary, pressure that modulates the amplitude of response of ecophysiological traits and likely the long-term population dynamics of native resprouting species.

The resource allocation theory predicts that plants growing in resource-limited environments should display lower growth and higher defenses against herbivores than plants growing under less limited resources (Boege and Dirzo 2004). However, while both species showed decreased SLA, it did not affect herbivory levels in *Desmodium*, but significantly increased herbivory in *Rhynchosia*. Our results imply that increased physical hardness of leaves did not represent a higher defense against herbivores. High levels of herbivory by grasshoppers in burned sites may be explained by their high mobility and their generalist feeding habits, which allows them to quickly colonize the burned areas from nearby unburned areas, being potentially resilient to recurrent fires (Swengel 2001; Kral et al. 2017; Koltz et al. 2018; Giorgis et al. unpubl. res.). Despite lower SLA levels, leaf nutrient concentration (N and P) was significantly higher in more frequently burned sites, which may attract herbivores (Carbone and Aguilar 2016). In agreement with previous studies, changes in floristic and nutritional composition may be strong drivers of the feeding patterns and population density of phytophagous insect in fire-prone environments (e.g. Christensen 1977; Reich et al. 1990; Rieske 2002; Adams and Rieske 2003; Kay et al. 2007; Carbone et al. 2017). Interestingly, the higher herbivore pressure in *Rhynchosia* plants growing in the highest fire frequency sites, it was not detrimental for their reproduction. Such results indicate a high herbivory tolerance without consequences to the probability of setting fruits and seeds in frequently burned sites.

Both plant species received similar pollinator visitation frequency across burned and unburned sites. These results imply their generalist bee pollinators were able to recolonize burned areas after the 3 years elapsed from the last fire event, providing similar pollination services as unburned sites (Carbone and Aguilar 2017; Peralta et al. 2017). A recovery of pollinator richness and pollination levels but altered composition has been found in recurrently burned sites (Lazarina et al. 2017) and after a single fire event in Mediterranean ecosystems (García et al. 2017). Thus, we may expect contrasting responses to fire frequency of different pollinator species depending on their feeding habits (e.g., specialist vs generalist). Plant species with broad pollinator assemblages or mostly generalist pollinators are more likely to ensure plant reproductive success in burned sites (Peralta et al. 2017). We found that similar pollination levels did not prevent reductions in the reproduction of *Desmodium*. Therefore, while fire may promote or not affect the diversity and abundance of pollinator in several ecosystems across the world (Peralta et al. 2017; Carbone et al. 2019), plant reproduction and their offspring performance may decrease due to reduction in abiotic resources and outcrossing rates following frequent fires, especially in exogamous species (LoPresti et al. 2018; Marquez et al. 2019).

Pre-dispersal seed predation in *Rhynchosia* showed comparable levels across sites with different fire frequency but equal time after the last fire. These results contrast to our initial predictions that fire frequency affects more drastically specialist interacting species. One possibility for not finding fire-frequency effects may be related to a low statistical power of observing these interactions on 6–12 plants per site coupled with the natural actual low frequency of seed predation. The few studies evaluating specialist seed predators of Fabaceae species in burned environments show varied responses among fire-prone regions. While seed damage in a Mediterranean ecosystem was lower in burned sites (García et al. 2016), postfire predation levels were comparable to unburned scenarios in two Australian shrubs (Auld and O'Connell 1989). Feeding and oviposition behaviour of seed predators can be affected by many different factors at the microsite scale. Plant size and flower production variations induced by spatial heterogeneity of burning within a site may be responsible for the recovery of seed predators (Cariveau et al. 2004; Carbone and Aguilar 2016). In synthesis, more studies need to be conducted to test whether specialization in plant–insect interactions represents a susceptible trait of the species to increased fire frequency.

Regarding seed predation interaction, while the presence of *Acanthoscelides* spp. has already been reported in congeneric species of *Rhynchosia* (Kingsolver 2004), this is the first formal report of a species-specific seed predator that represents the main antagonism affecting the progeny of *R. edulis*. The seed beetle *Acanthoscelides* sp. was not found in other coexisting legume species throughout all sites, therefore, *Rhynchosia-Acanthoscelides* is an interesting system to continue inquiring about the response of specialist biotic interactions to anthropogenic changes in fire regime. The analysis of male genitalia of this bruchid species did not agree with any of the described *Acanthoscelides* species (Johnson 1990; Kingsolver 2004; Terán 2013, pers. comm.). Therefore, as for a large number of species of this genus, the identity of this bruchid species remains to be described and needs future taxonomic, ecological and genetic studies (Johnson 1990).

In conclusion, we report that resprouting herbs in frequently burned sites can decrease their sexual reproduction by abiotic constraints in relation to unburned habitats. However, within burned areas, these species efficiently maximized the allocation to reproduction expressing a

conservative strategy in the use of resources when SLA levels are extremely low. This is an interesting aspect that should be inspected in other resprouting herbs with different types of underground storage organs. Pollination and seed predation appeared to recover after three postfire years in repeatedly burned sites regardless of the level of specialization of the interacting insects. Increased leaf herbivory in high fire frequency habitats had no reproductive consequences for *Rhynchosia,* which appears to be tolerant to high biotic stress. Adult individuals of *Desmodium* and *Rhynchosia*, like other widespread plant species from Chaco Serrano, resprout successfully after the fire and exhibit tolerance to recurrent disturbances However, decreased plant fecundity, especially in *Desmodium*, is likely to impact its local soil-seed bank, reducing seedling recruitment, and potentially affecting the density and long-term viability of populations growing in high fire frequency areas. Our results highlight the ecological importance of assessing vegetative traits and biotic interactions as drivers of plant reproductive dynamics in scenarios anthropogenically perturbed by fire frequency.

Data availability All data generated and analysed during this study are included in the supplementary information files.

Code availability The authors declare that they have used free software to statistical analysis (R) and the scripts are available in the supplementary information.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval We have to the permission of the proprietors of fields and the nature reserve authorities for the field sampling.

Consent to participate The authors declare that they have consented to participate in this paper.

Consent for publication The authors declare that they have consented to the publication of this manuscript.

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