Germination characteristics of five coexisting neotropical species of *Acacia* in seasonally dry Chaco forests in Argentina

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Abstract

At a regional scale, habitat heterogeneity can play a very important role in allowing the coexistence of species through their specialization in the use of patches differing in environmental conditions. Here, we explore responses of seed germination to environmental and anthropogenic factors of five *Acacia* species that co-occur in subtropical dry Chaco forests in central Argentina and the habitat heterogeneity of this region. Open and closed sites were characterized in terms of environmental factors by measuring temperature, water content, light quality and soil conditions. Through germination experiments, we evaluated seed responses to both environmental (temperature, light quality and water stress) and anthropogenic factors (fire and cattle). Data were analyzed through a univariate analysis (generalized linear model, GLM) followed by a multivariate analysis (principal component analysis, PCA) in order to obtain a general pattern of *Acacia* seed responses. Some differences were detected among the *Acacia* species at the species level and the multivariate analysis (PCA) showed a general pattern with two groups of species differing in germination characteristics: *A. aroma*, *A. caven* and *A. atramentaria* had a higher number of hard seeds after fire and seed consumption by cattle, whereas *A. praecox* and *A. gilliesii* showed a reduced time to germinate under different environmental conditions. The multivariate analysis suggests a regenerative niche differentiation between two groups of *Acacia* species. The habitat heterogeneity described in the dry Chaco forests from central Argentina could facilitate the coexistence of the five *Acacia* species at the landscape and regional levels.

Keywords: Acacia, niche differentiation, seed germination, species co-existence, subtropical dry forests.

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Introduction

One of the most critical stages in the life cycle of a plant is germination, a key process in sexually reproducing plants (Poorter 2007). Germination can occur under a great variety of environmental conditions because species maximize establishment and survival of new sexually produced individuals in different abiotic and biotic contexts (Baskin & Baskin 2014). Temperature, light and moisture are among the most important environmental factors influencing and controlling this stage (Baskin & Baskin 2014). These factors may vary as a consequence of past and present changes in the vegetation cover due to both natural and anthropogenic causes. Such changes have occurred in many regions worldwide, including the Chaco seasonally dry forests of central Argentina, currently consisting of a mosaic of vegetation patch types, from closed (old grown forests, woodlands and tall shrublands) to open habitats (open low shrublands and low-cover scrub vegetation) (Cabido *et al.* 1994; Zak & Cabido 2002). For example, changes in plant cover may

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alter the intensity and quality of the light reaching the topsoil (Federer & Tanner 1966), and may also modify the temperature regime and moisture availability at the soil surface, by promoting extreme temperatures (Aguiar & Sala 1999). Consequently, environmental conditions in different vegetation patch types may differ substantially (Bullock 2000), possibly affecting the germination patterns of seeds (Baskin & Baskin 2014). Therefore, species inhabiting a given patch type are expected to have more similar germination characteristics than species from a different patch (Chase & Leibold 2003; Leibold *et al.* 2004). Habitat heterogeneity resulting from the spatial and temporal variation of abiotic factors, as well as other causes such as fire and herbivory (Stewart *et al.* 2000), may play a very important role in species distribution, especially at the landscape scale where patches differing in environmental conditions are likely to occur (Kneitel & Chase 2004). Fire and endozoochory, factors that can be natural or human induced, can favor the loss of impermeability to water in seeds with physical dormancy, thus promoting germination (Auld & O' Connell 1991; Peco *et al.* 2006).

Studies of recruitment from seeds in the Chaco are scarce, complicating the assessment of regeneration strategies after disturbance (Jaureguiberry & Díaz 2015). Barchuk *et al.* (1998) reported significant seedling recruitment of *Aspidosperma quebracho-blanco* (Apocynaceae) in protected areas (closed habitats) of the western Chaco, and little recruitment in areas with extensive grazing and uncontrolled logging (open sites or gaps). The fire tolerance of seeds of Chaco woody species could have evolved as a consequence of the evolutionary pressure exerted by other factors, a phenomenon called 'exaptation' (Gould & Vrba 1982). Specifically, the presence of hard seed coats, a trait of adaptive value in endozoochorous species dispersed by ungulates (Peco *et al.* 2006) or subjected to seasonal desiccation (Baskin *et al.* 2000) (both common in the Chaco region), could be an exaptation giving the seeds a high tolerance of heat. Five neotropical species of *Acacia*, *A. aroma* Guilles ex. Hook. & Arn., *A. caven* (Molina) Molina, *A. atramentaria* Benth, *A. gilliesii* Steud. and *A. praecox* Griseb., co-occur in this heterogeneous mosaic; at the local scale, the abundance of the five species varies between open and closed sites (Cabido *et al.* 1994, Table 1 in Appendix S1). If germination of seeds is often restricted to locations that meet specific environmental conditions ('safe sites'), it may be expected that species inhabiting different patches will show divergences in certain germination characteristics, which increase their suitability to the conditions prevailing in a given habitat. In addition, if the species involved in the comparison show similar physiological, morphological and anatomical traits at the adult stage (Cialdella 1984; Díaz & Cabido 1997), it may be predicted that they will differ at the regenerative phase (i.e. in dormancy-

breaking by anthropogenic factors and germination requirements attributed to habitat adaptations) (Grubb 1977; Chase & Leibold 2003; Vandelook *et al.* 2008) and that the chance of dormant seeds developing into successful seedlings will depend on the environmental conditions at the local scale.

In this paper we analyzed the germination response to fire and endozoochory, and the germination characteristics, of five sympatric Neotropical *Acacia* species that coexist in the dry Chaco forests of central Argentina. Our hypothesis is that the species co-occurring at open forest and shrubland sites will differ in their germination characteristics from species that co-occur at closed canopy sites. It is noteworthy that there are not too many studies that have followed this approach to relate germination strategies to habitat conditions at the species level (e.g. Daws *et al.* 2002; Van Assche *et al.* 2002; Vandelook *et al.* 2008; Oliveira & García 2011).

Materials and methods

Study area

In central and northern Argentina, lowlands filled with quaternary sediments are occupied by seasonally dry subtropical Chaco forests. Potential natural vegetation in this area comprises seasonally dry xerophytic forests and woodlands dominated by *Aspidosperma quebracho-blanco* (Apocynaceae), but at least two centuries of logging, fire and grazing by introduced herbivores have transformed plant cover into a mosaic of low forests, woodlands, shrublands and scrubs, where rather isolated patches of closed forests alternate with open habitats with a high proportion of bare soil (locally known as 'peladares': sites with very low plant cover) (Cabido *et al.* 1994; Zak & Cabido 2002; Jaureguiberry & Díaz 2015). The climate in the area is warm temperate to subtropical with summer rains. Mean annual rainfall ranges between 500 and 600 mm, and mean annual temperature is 19.9°C (Capitanelli 1979).

Studied species and seed collection

The studied species, *Acacia aroma*, *A. caven*, *A. atramentaria*, *A. gilliesii* and *A. praecox*, are shrubs or small trees that occur in arid and semiarid Neotropical regions at 0–1500 m a.s.l. These five *Acacia* species are sympatric in seasonally dry subtropical Chaco forests in Córdoba, central Argentina. The five species of *Acacia* are very similar in vegetative traits at the adult stage, have similar physiological, anatomical and morphological adaptations, and have a similar distribution range at the regional scale (Cialdella 1984; Díaz & Cabido 1997). Therefore, the most important ecological difference among these species occurring in the dry Chaco is their

local habitat preference, because *Acacia aroma* and *A. caven* are characteristic of open and disturbed forests, whereas *A. gilliesii* and *A. praecox* are more frequent in closed and mature forests. *Acacia atramentaria* occurs in both open and closed forests but tends to be more frequent in open habitats (Cabido *et al.* 1994; Zak & Cabido 2002. Table 1 in Appendix S1).

Between 2006 and 2009, mature dry seeds were collected from 20 individuals of each of the five species in north-western Córdoba (30°45′32″S, 64°55′30″W), at about 600 m a.s.l. Seeds were stored in paper bags at room temperature for 30 days before the start of the experiments.

Environmental factors

Ten sites of at least 5 ha each were selected within the study area; five sites were open (with evident signals, for example presence of tree stumps, large areas of bare soil and feces of cattle, of anthropogenic disturbance, such as fire, deforestation and livestock grazing) and the other five had a closed canopy, representing well-conserved forests (Cáceres *et al.* 2015). The following environmental factors were measured to describe the 10 sites: soil temperature, soil water content, soil pH, soil texture, soil organic matter content, organic carbon and light quality. Measurements were taken from May 2008 to May 2009, both under the canopy of shrubs and between shrubs (open gaps) at the open sites and under the tree canopy at the closed sites.

Soil temperature was measured using three sensors (iButton Viewer32) at each closed site and six sensors (three under the canopy of the shrubs and three in open gaps) at each open site. The sensors were set up to record temperature daily every 2 h and they were buried at not more than 5 cm depth. Soil water content was measured using a moisture probe meter (MPM, 160-B). Two measurements were taken in each of the four seasons, each consisting of five records at the closed sites and 10 records (five under the canopy and five in open gaps) at each open site. Light quality (Red and Far Red) was measured at the level of the soil surface twice per season (five records at each closed site and 10 records [five under the canopy and five in open gaps] at each open site), using a Red:Far Red sensor (Skye, Instruments Ltd). Soil samples were collected in order to measure texture, pH, organic carbon and organic matter content. A composite sample comprising 10 subsamples was collected at each site. Soil analyses were performed at the Soil and Water Laboratory of the Agricultural Sciences Faculty (National University of Córdoba).

Germination experiments

Responses to environmental factors: temperature, water stress and light quality Three germination experiments were conducted to evaluate seed responses to temperature, water stress and light quality. For each trial, three replicates of 25 seeds each were used. The seeds were placed in Petri dishes on filter paper soaked in distilled water and then incubated in germination chambers equipped with fluorescent tubes of 20W cool white light with a radiation density (400–700 nm) of about 38 μm m^{-2} s^{-1}. Seeds of the five species were scarified with sandpaper because three of them have physical dormancy (*A. aroma*, *A. caven* and *A. atramentaria*; Funes & Venier 2006; Venier *et al.* 2012). Seeds were kept in the germination chamber for 30 days. The number of germinated seeds was counted daily, except in the permanent darkness treatment (see below), for which germination was recorded at the end of the incubation period. The germination criterion was radicle protrusion of at least 2 mm.

To evaluate seed response to temperature, germination tests were performed in germination chambers under a 12-h photoperiod and under continuous darkness (Petri dishes wrapped with aluminum foil) using four thermoperiods: 15/5°C, 20/10°C, 25/15°C and 35/20°C; thermoperiods were established to simulate the average temperature ranges common to each season of the year in the study area (Capitanelli 1979). To evaluate the response to water stress, seeds were subjected to five osmotic potentials at 25/15°C: 0.0 (control), −0.35, −0.75, −1.2 and −1.5 MPa. Each potential was generated using polyethylene glycol 6000 (PEG) at different concentrations (Michel & Kaufmann 1973).

To evaluate the response to different light quality conditions, seeds were exposed to different R:FR ratios. Petri dishes containing the seeds were placed in a plastic container (35 cm × 25 cm × 10 cm) covered with two red filters (Lee N° 106); thus, seeds received red light of 600–730 nm of energy with an R:FR between 3.97 and 4.05, which was equivalent to the values recorded at the open sites in the study area (see Results). Another set of three Petri dishes per species were placed in a plastic container but now covered with a red filter plus a blue filter (Lee N° 183); these seeds then received far red light of 600–940 nm of energy with an R:FR between 0.24 and 0.32 (Yirdaw & Leinonen 2002). This R:FR ratio was equivalent to the values recorded at the closed sites of the study area. Another group of seeds was incubated under white light as a control treatment.

Responses to simulated fire and herbivore consumption

Simulated fire experiment An experiment was conducted to assess the effect of fire on seed germination (experimental heat shock). For each species, three replicates (30 seeds each) were placed in an oven at 90°C and 190°C, during two time periods, 1 and 5 min; thus, four

treatments were considered: (i) 90°C 1′, (ii) 90°C 5′, (iii) 190°C 1′, (iv) 190°C 5′. Time and temperature combinations were selected on the basis of available records of temperature and duration of simulated fires in an ecosystem similar to the one in the study area, and according to data from the literature (Hanley & Lamont 2000; Hanley *et al.* 2001; Ne'eman *et al.* 2009; Jaureguiberry & Díaz 2015). Seeds were put in an aluminum container containing a substratum of soil and sand. After exposure in the oven, the seeds were taken to germination chambers, where they were incubated in Petri dishes with filter paper soaked in distilled water at 25/15°C, 12-h/12-h light/darkness for 30 days. A control of three replicates (15 untreated seeds each) for each species was also incubated in the germination chamber under the same conditions.

Seeds were observed daily and the percentages of germinated, dead and hard seeds were recorded at the end of the experiment. Seeds that had not germinated at this stage of the experiment were scarified with sandpaper and returned to the germination chambers for 15 days.

Simulated herbivore consumption experiment Seeds of the five *Acacia* species were subjected to a simulated herbivore consumption treatment, by exposing them to chemical attack by both ruminal and acid digestion, and then set to germinate. Because the seeds of these species are likely to pass unharmed through the digestive tract of ruminants, as reported for other legumes with similar fruits (Peinetti *et al.* 1993), they were removed from the pods before they were subjected to the herbivory experiment. Three replicates (40 seeds each) of the five species were placed in heat-sealed nylon mesh bags. The mesh allowed free passage of microorganisms. All bags were incubated for 48 h inside the rumen of three fistulated cows (Peco *et al.* 2006) in the 'Estación Experimental Manfredi', National Institute of Agricultural Technology (INTA).

Seeds are usually retained in the most acid part of the gut (abomasum and duodenum) for 2–4 h (Warner 1981); hence, after removal from the rumen, each bag and its contents were rinsed with tap water and then loose seeds (without the bag) were immediately placed in a 0.1 N pepsin-hydrochloric acid solution in an oven at 40°C for 2 h. The solution was prepared by dissolving 1 g of pepsin in 1 L of HCl 0.1 N (Peco *et al.* 2006). After both ruminal and acid digestion, and before incubation in germination chambers, the numbers of germinated, imbibed (permeable, ungerminated seeds that lost physical dormancy), hard (intact, firm, ungerminated and impermeable seeds, still with physical dormancy) and dead (rotten and digested) seeds were recorded. After both ruminal and acid digestion, imbibed and still hard seeds, and three control seed replicates (each replicate consisting of 20 seeds that had not been subjected to the digestive processes), of the five *Acacia* species were set to germinate for 30 days in growth chambers with 12-h/12-h light/dark at 25/15°C. Replicates were placed in Petri dishes on filter paper saturated with distilled water. After incubation at 25/15°C, the numbers of germinated, still hard and rotten (or digested) seeds were recorded. All seeds that remained hard after incubation at 25/15°C were scarified with a razor blade and returned to growth chambers for 15 days. Germination of scarified seeds was recorded at the end of the experiment.

Statistical analysis

Mean time to germination (MTG) for seeds of the five species under different conditions of temperature, light and water potential (0.00 and −0.35 Mpa) was calculated using the following equation (Daws *et al.* 2002):

MTG = $\sum$ (n × d)/N, where *n* is the number of germinated seeds between count intervals, *d* is the incubation period in days at each count time and *N* is the total number of germinated seeds in a given treatment.

One-way ANOVA and *post hoc* Fischer's LSD test were used to compare soil variables (soil characteristics measured from the soil samples) between open and closed sites. Records of light quality were Ln-transformed to meet the assumptions of homogeneity of variance. An ANOVA with repeated measures was performed to compare seasonal variability of water content and light quality.

A generalized linear model (GLM) with a logit link function for binomial distribution was used in order to analyze the effect of environmental factors on seed germination (Bolker *et al.* 2008). The same GLM was used to analyze the effect of simulated fire on germination where the dependent variables were the numbers of germinated, hard and dead seeds after the simulated fire experiment considering two factors, Species and Fire, with different combinations of temperature and time + control. For the same experiment, another GLM was performed to analyze the number of scarified seeds that germinated, followed by a *post hoc* Di Rienzo, Guzmán and Casanoves (DGC) test (Di Rienzo *et al.* 2002; Table 2 in Appendix S1).

After the simulated herbivore consumption experiment, a Friedman test was used to compare the percentage of hard (intact) and soft (imbibed) seeds among species. A *t*-test was performed to compare final germination percentages of treated and control seeds within each species (Sokal & Rohlf 1995).

In order to compare the responses of the different species within each treatment, mean time to germination (MTG) was analyzed through an ANOVA and *post hoc* Fischer's LSD test. A Kruskal Wallis test was used in cases in which the ANOVA assumptions were not met. The variable MTG at 0.00 MPa was log-10 transformed with the aim of meeting the ANOVA assumptions (Sokal & Rohlf 1995).

A principal component analysis (PCA) was performed with all the variables (those related to responses to environmental factors [temperature, water stress and light quality], those related to anthropogenic factors [fire and livestock] and the MTG variables, a total of 40 variables) to identify a general pattern of similarity among species and to identify the variables that best explained that pattern. All the analyses were performed using Infostat version 2013 (Di Rienzo *et al.* 2013).

Results

Environmental factors

The closed canopy sites (well-conserved forests) had higher percentages of organic matter and organic carbon than the open sites (shrublands and scrubs resulting from anthropogenic disturbance) but soil pH and soil texture did not differ between habitats (Table 1).

The R:FR ratio was higher at open than at closed sites; within open sites, it was even higher in open gaps than under the canopy (Fig. 1), with significant statistical differences between sites ($F = 333.19$; $P < 0.0001$) but not among seasons ($F = 1.727$; $P = 0.211$). The season–site interaction was also statistically significant ($F = 7.978$; $P = 0.002$). The R:FR ratio increased in winter at closed sites and under the canopy at open sites due to loss of tree leaves in this season, but decreased in open gaps. Moreover, in spring the R:FR ratio increased in open gaps and decreased at closed sites and under the canopy at open sites (Fig. 1). Soil water content was higher at open sites than at closed sites ($F = 18.93$; $P = 0.0002$) (Fig. 1). Within open sites, no significant differences were found between the subtypes 'open gaps' and 'under the canopy'. Soil water content varied seasonally for both habitat types ($F = 34.996$; $P < 0.0001$), but no interactions were detected between sites and seasons ($F = 0.597$; $P = 0.745$). Following the regional precipitation patterns, soil water content was higher during spring and summer than during autumn and winter (Fig. 1). Soil temperature varied between sites: mean temperature and thermal amplitude were higher at open than at closed sites, and within the former, both variables showed higher records in open gaps than under the canopy (Fig. 2).
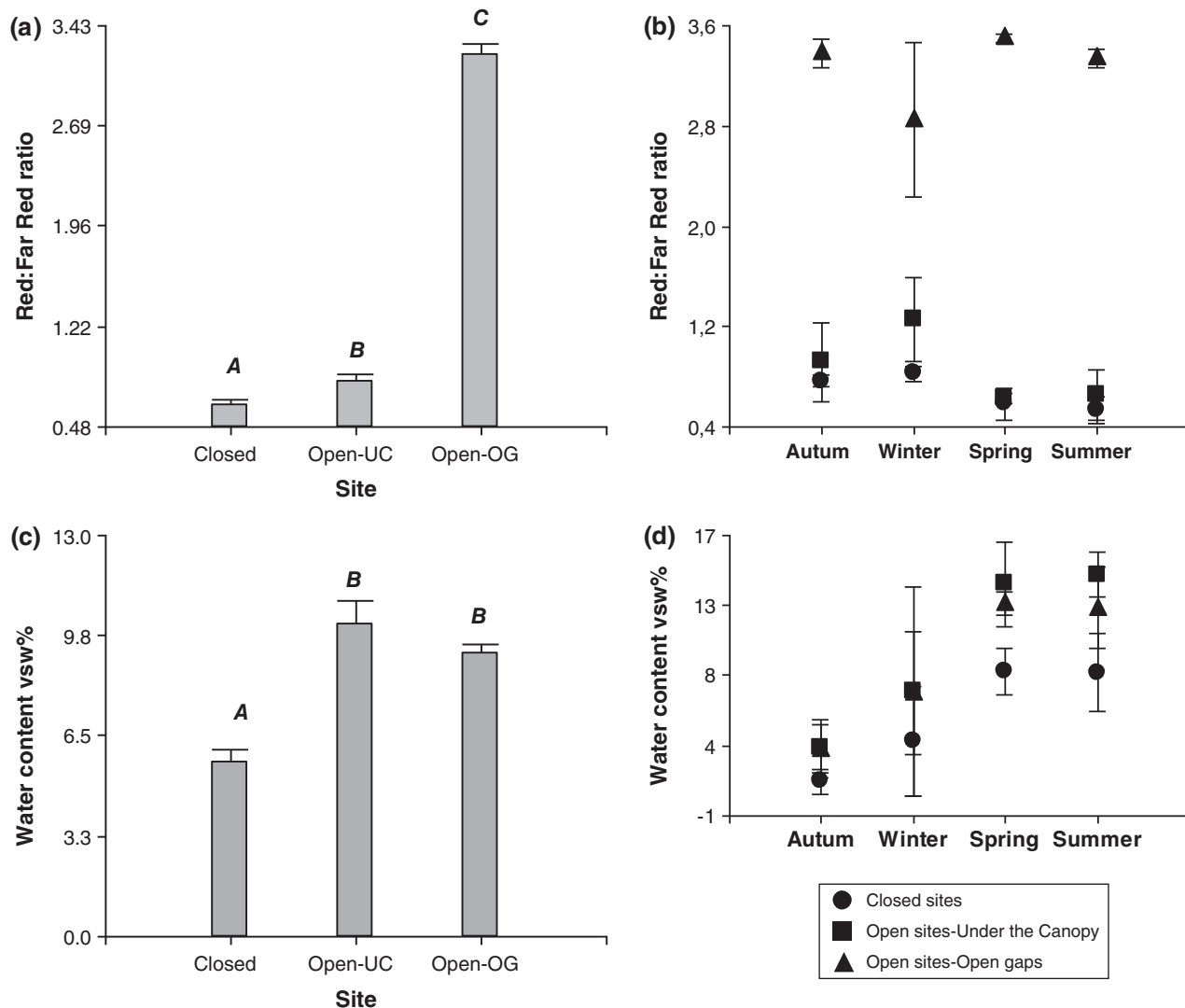
Germination experiments

Responses of seed germination to environmental factors: temperature, water stress and light quality The effects of the factors Species, Temperature and Light/Dark, and all of the double interactions, on the number of germinated seeds were statistically significant ($P < 0.0001$). However, the triple interaction was not statistically significant ($P = 0.7893$). All species showed high percentages of germination at the three highest temperatures (35/20°C, 25/15°C, 20/10°C) both under light and in permanent dark. However, at the lowest temperature (15/5°C), only *A. gilliesii* and *A. praecox* germinated successfully, both under light and dark conditions (Table 2). *Acacia aroma* had the lowest germination percentage in the dark (Table 2). All species exhibited high germination percentages at 0.0 and −0.35 MPa, but germination was very low or nil at the other water potentials, with *A. praecox* and *A. gilliesii* showing a trend towards a higher capacity for germination at more negative water potentials than the other three species (Table 2). The effect of water potential was statistically significant ($P < 0.0001$; factor Species, $P = 0.1497$), but the effect of the interaction between species and water potential on the number of germinated seeds was not statistically significant ($P = 0.1168$). All of the five species had high germination percentages at the three light-quality conditions (white, red and far red) (Table 2). Only the factor Species had a statistically significant effect on the number of germinated seeds ($P = 0.0008$; factor Light, $P = 0.6097$; interaction of factors, $P = 0.1375$).

Germination rate In general, *A. praecox* was the species with the highest germination rate within each treatment, followed in decreasing order by *A. glliesii*, *A. aroma*, *A. caven* and *A. atramentaria*. The differences in MTG among species were statistically significant (Fig. 3).

Responses of seed germination to anthropogenic factors: fire and livestock

Simulated fire experiment The effects of factors (Species and Fire) and their interactions on the numbers of germinated ($P < 0.0001$) and dead seeds ($P < 0.0001$) were

Table 1 Differences (mean ± SE) in soil characteristics between open and closed sites in subtropical Chaco forests from central Argentina

Site	Organic matter (%)	Organic carbon (%)	pH	Clay (%)	Silt (%)	Sand (%)
Open	2.30 ± 0.27	1.34 ± 0.16	7.06 ± 0.14	12.24 ± 1.64	21.94 ± 2.18	65.82 ± 3.50
Closed	3.1 ± 0.20	1.8 ± 0.11	7.13 ± 0.05	15.2 ± 0.58	25.66 ± 3.42	59.14 ± 3.42
F	5.65	5.48	0.2	2.88	0.84	1.86
P	0.0447*	0.0474*	0.6642	0.128	0.3855	0.2098

Asterisks indicate significant differences ($P \leq 0.05$, Fisher's LSD test) between sites.

Fig. 1 Light quality (Red:Far Red; R:FR) (a, b) and soil moisture (c, d) at open and closed sites in subtropical Chaco forests in central Argentina (bar graphs; different letters indicate significant differences ($P < 0.05$, Fisher's LSD). UC, under the canopy; OG, open gaps.

statistically significant. Only the effect of the factor Species on the number of hard seeds was statistically significant ($P < 0.0001$; factor Fire, $P = 0.7603$; interaction of factors, $P = 0.7963$).

Most seeds of *A. aroma*, *A. caven* and *A. atramentaria* remained hard after the simulated fire experiment for all combinations of temperature and time (Fig. 4). When these seeds were scarified and returned to incubation, the germination response varied depending on the heat treatment: at 190°C 5′ only a few seeds germinated and the remaining ones died for all three species; whereas under the other treatments (190°C 1′, 90°C 5′ and 90°C 1′) the three species showed high percentages of germination (Table 2 in Appendix S1). For the other combinations of time and temperature (190 °C 1′, 90 °C 5′ and 90 °C 1′), scarified seeds of these three species had high

germination percentages (Table 2 in Appendix S1). *Acacia gilliesii* and *A. praecox* had high percentages of dead seeds at 190°C 5′, whereas with the other combinations of temperature and time, these two species had high germination percentages (Fig. 4). The seeds that remained hard and then were scarified reached high germination percentages (Table 2 in Appendix S1). For scarified seeds, the factors and their interactions were statistically significant ($P < 0.0001$).

Simulated herbivore consumption experiment *Acacia aroma* exhibited a statistically significantly higher germination percentage (71.5%; $t = -11.85$, $P = 0.0001$) after the entire treatment (ruminal digestion + acid digestion + incubation chamber); this result is in contrast with the control germination of 6.7%. Of the remaining seeds, 9.19% died
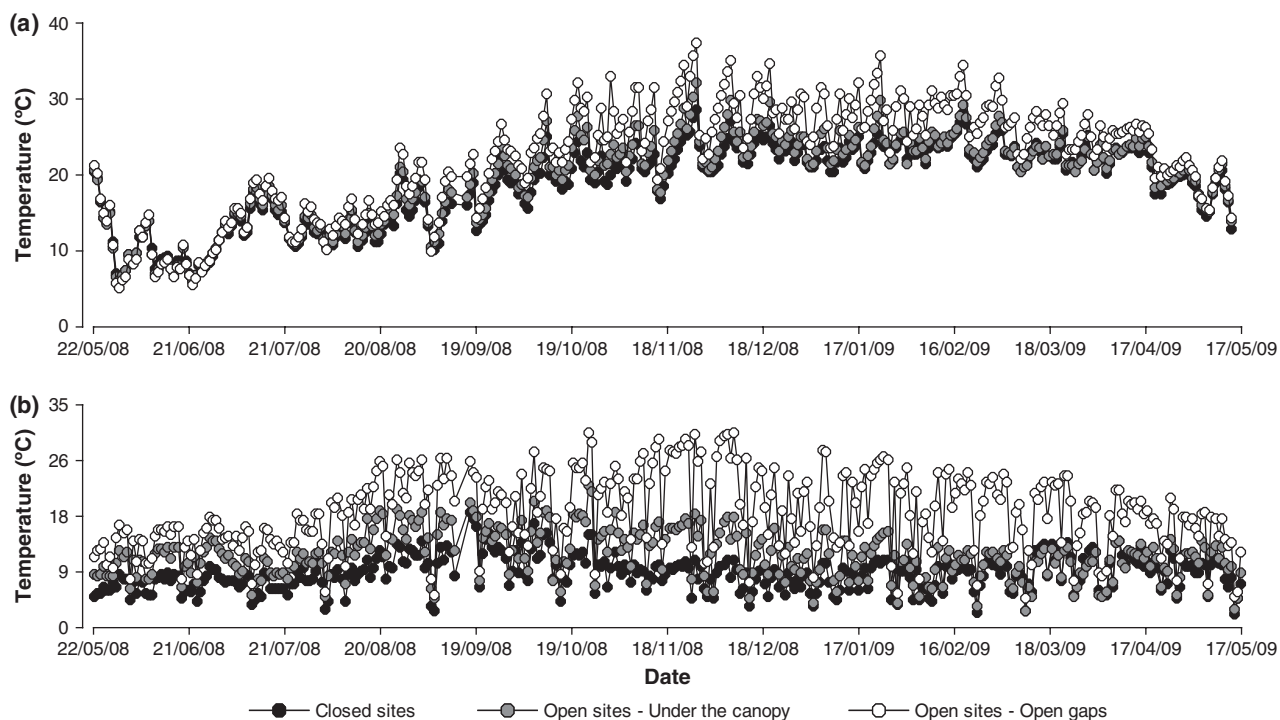
Fig. 2 (a) Soil daily mean temperature and (b) soil daily thermal amplitude at closed and open sites in Chaco forests in central Argentina.

Table 2 Mean germination percentage (± SE) of five *Acacia* species during four thermoperiods under light and dark conditions, under five different water potential conditions and under three different light-quality conditions

	Germination (%)				
	A. aroma	*A. caven*	*A. atramentaria*	*A. gilliesii*	*A. praecox*
Temperature (°C), light					
35/20	94.67 ± 2.67	97.33 ± 1.33	88 ± 0	100 ± 0	100 ± 0
25/15	70.67 ± 2.67	94.67 ± 2.67	92 ± 0	98.67 ± 1.33	98.67 ± 1.33
20/10	81.33 ± 5.33	92 ± 6.11	92 ± 4	100 ± 0	100 ± 0
15/5	11.67 ± 6.01	5.33 ± 1.33	2.67 ± 1.33	92 ± 2.31	96 ± 2.31
Temperature (°C), dark					
35/20	92 ± 2.31	100 ± 0	96 ± 4	100 ± 0	100 ± 0
25/15	52 ± 8	97.33 ± 1.33	92 ± 4.62	98.67 ± 1.33	100 ± 0
20/10	16 ± 2.31	94.67 ± 1.33	84 ± 4	98.67 ± 1.33	100 ± 0
15/5	0 ± 0	0 ± 0	0 ± 0	52 ± 8	70.67 ± 5.81
Water potential (MPa)					
0.0	97.33 ± 1.33	97.33 ± 1.33	97.33 ± 2.67	100 ± 0	100 ± 0
−0.35	98.67 ± 1.33	93.33 ± 2.67	92 ± 2.31	100 ± 0	97.33 ± 2.67
−0.75	0 ± 0	0 ± 0	2.67 ± 2.67	9.33 ± 1.33	32 ± 6.11
−1.20	0 ± 0	0 ± 0	0 ± 0	1.33 ± 1.33	0 ± 0
−1.75	0 ± 0	0 ± 0	0 ± 0	1.33 ± 1.33	0 ± 0
Light					
White	97.33 ± 1.33	97.33 ± 1.33	98.67 ± 1.33	100 ± 0	100 ± 0
Red	98.67 ± 1.33	100 ± 0	90.67 ± 3.53	100 ± 0	100 ± 0
Far red	97.33 ± 1.33	100 ± 0	97.33 ± 1.33	98.67 ± 1.33	100 ± 0

See text for more details.

and 19.36% remained impermeable (Fig. 5). Seeds of *A. atramentaria* and *A. caven* remained almost entirely impermeable after ruminal + acid digestion. In addition, the seeds of *A. atramentaria* that were soft (imbibed) after both ruminal and acid digestion died during incubation in the germination chamber. The controls for
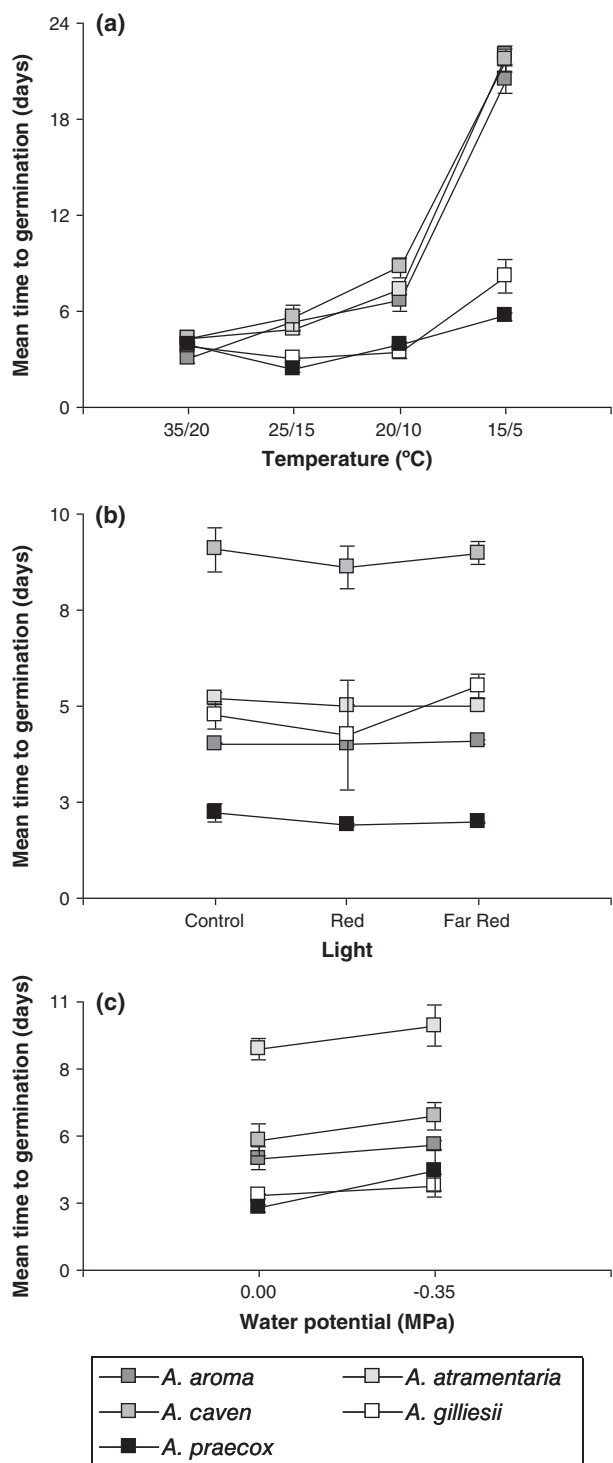
Fig. 3 Germination rate expressed as mean time to germination (MTG, days) for five *Acacia* species and under different conditions of (a) temperature, (b) light and (c) water potential.

A. atramentaria and *A. caven* showed a very low germination percentage (1.67%), which was not statistically different from the nil germination percentage of the treated seeds ($t = 1$; $P > 0.05$). *Acacia praecox* exhibited a very low germination percentage (1.71%) after the entire treatment (ruminal digestion + acid digestion + incubation chamber), which contrasts with the control germination of 98.33% ($t = 41.01$; $P = 0.0001$). The remaining treated seeds (98.29%) died (Fig. 5). Some seeds of *A. gilliesi* germinated (50.83%) after the entire treatment but the percentage was not statistically different from the 61.67% of germination of the control seeds ($t = 1.54$; $P > 0.05$). Of the remaining seeds, 27.5% died and 21.67% remained impermeable (Fig. 5). In all the species, both control and treated seeds that failed to germinate and were then scarified reached high germination percentages (88–100%), confirming high seed viability after treatment; *A. praecox* was an exception, because this species had a high percentage of dead seeds after treatment.

Principal component analysis

The first axis of the PCA explained 60.7% of the variation and discriminated two groups of species: one comprising *A. aroma*, *A. caven* and *A. atramentaria* and the other comprising *A. praecox* and *A. gilliessi* (Fig. 6). The variables that best explained this pattern were those related to MTG, germination capacity under water stress and seed response to the simulated fire and cattle consumption experiment. The second axis explained 18.2% of the variation and separated the species within the group of *A. aroma*, *A. caven* and *A. atramentaria.* Germination after consumption by cattle and MTG at 35/20°C were the variables that best explained the pattern displayed along axis 2 (Fig. 6).

Discussion

Our results show that open sites differed from closed sites with respect to soil temperature, soil water content and light quality, providing evidence that the structural heterogeneity of the vegetation described in these forests results in different habitat conditions for plant species. In general, environmental conditions at open sites were found to be more extreme than those at closed sites: higher mean temperature, thermal amplitude and R:FR ratio in the open than in the closed habitats. Although soil water content values were very low at both sites, closed sites showed lower records than open sites. A similar result was found at other sites within the same Chaco region (Barchuck *et al.* 2005). Closed sites also had higher percentages of organic matter and organic carbon than open sites, most probably because plant cover is higher at closed sites, providing higher amounts of litter than at the open sites (Barchuck *et al.* 2005). Therefore, as expected, habitat heterogeneity induced by human disturbance is reflected in the variation of environmental
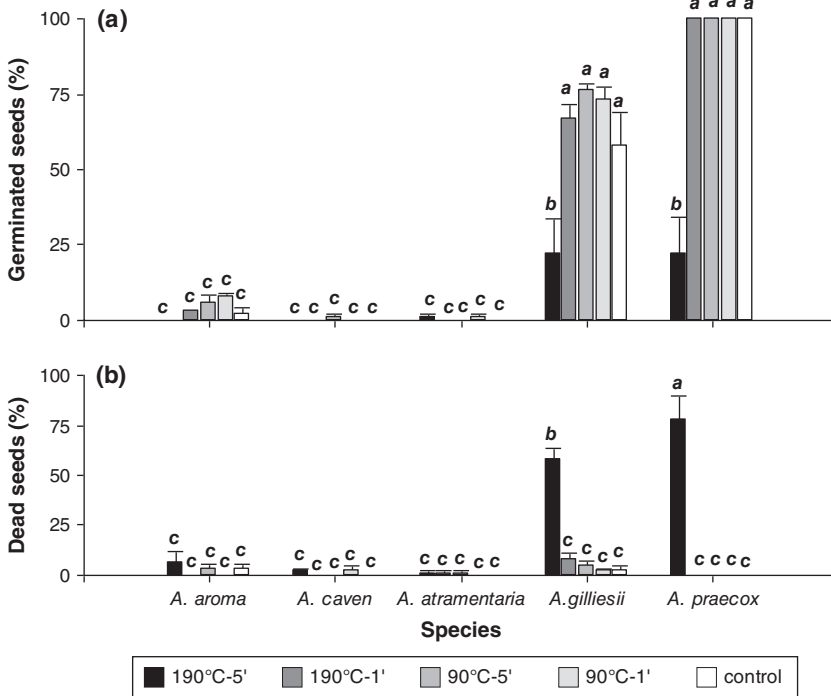
Fig. 4 Mean percentages of germinated (a) and dead (b) seeds after each simulated fire treatment (different combinations of temperature and time in an oven followed by incubation in a germination chamber at 25/15°C, 12-h/12-h light/dark) and control (untreated seeds incubated in a germination chamber at 25/15°C, 12-h/12-h light/dark). Seeds that did not germinate or die remained hard. Different letters indicate significant differences ($P < 0.05$, DGC test).
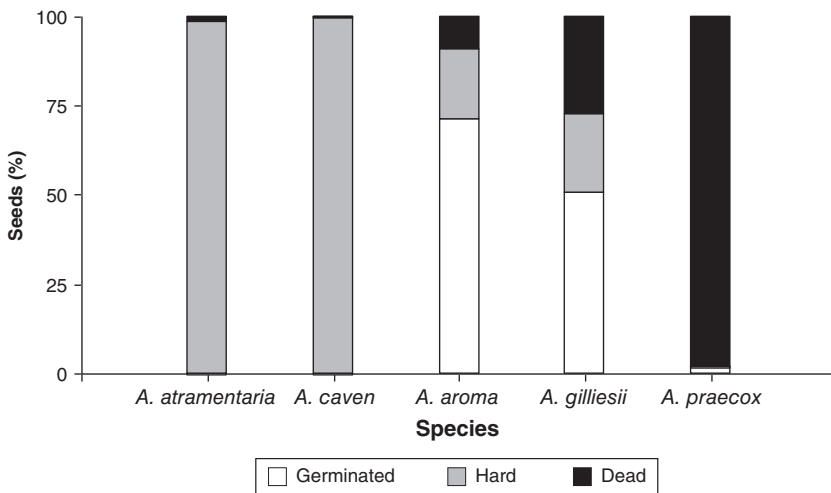

Fig. 5 Mean percentages of germinated, hard and dead seeds after ruminal digestion followed by acid digestion and subsequent incubation for 30 days in a germination chamber at 25/15°C, 12-h/12-h light/dark.

factors in the dry forests of Córdoba, as previously reported for other ecosystems in the world (Vandelook *et al.* 2008 and references therein). Dormancy-release mechanisms can be habitat dependent and a wide range of dormancy-release factors for seeds with physical dormancy have been proposed, for example high and fluctuating temperatures (van Klinker & Goulier 2013). In this sense, the *Acacia* species with physical dormancy studied here could be favored to break dormancy by the extreme and fluctuating temperatures registered at the open sites. However, further testing would be required to prove this hypothesis and to determine possible differences at the species level and/or population level.

Responses of seed germination to environmental factors: temperature, water stress and light quality

Some differences were observed in the germination requirements for temperature, light and water among the five *Acacia* species studied. All the species showed high germination percentages at 35/20°C, 25/15°C and 20/10°C, especially under light conditions; this germination pattern could be related to the seasonal rainfall regime (spring–summer rainfall) in the dry forests of Córdoba, as occurs with other species from the same ecosystem (Funes *et al.* 2009). However, *A. praecox* and *A. gilliessi* also showed high percentages of germination
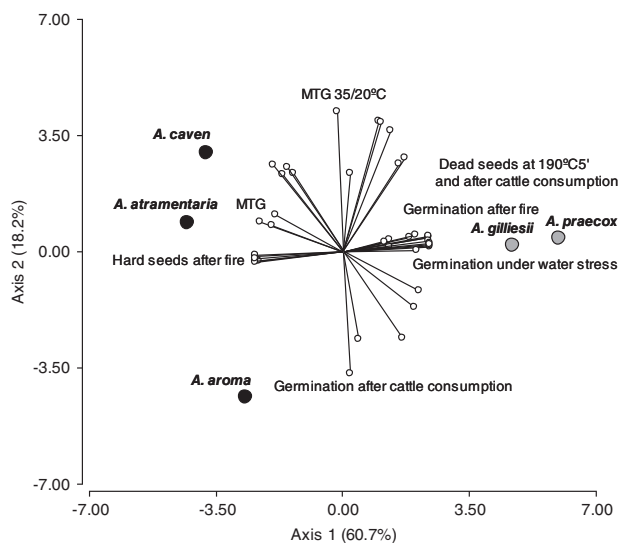
Fig. 6 Principal component analysis (PCA) biplot showing the variables most correlated with axes 1 and 2. Hard seeds after fire: summarizes vectors that indicate the percentage of hard seeds after the different fire treatments. MTG: summarizes the vectors that indicate mean time to germination at 25/15°C, 20/10°C, 15/5°C, −0.0 and −0.35 MPa. Germination under water stress: summarizes vectors that represent variables of germination at −0.75, −1.2 and −1.5 MPa. Germination after fire: summarizes vectors that represent germination variables in each fire treatment. Dead seeds at 190°C 5′ and after consumption by cattle: corresponds to two variables (vectors) that indicate percentage of dead seeds in these two treatments.

at the lower temperature (5/15°C); therefore, these two species would have greater ability to germinate at a wider temperature range than *A. aroma*, *A. caven* and *A. atramentaria*.

Our results also show that the five species need a high amount of water to germinate. However, *A. gilliesii* and *A. praecox* exhibited a trend to germinate at lower hydric potentials, indicating a higher tolerance of water deficit by these two species compared with the other three. This result could be related to the lower soil water content found at the closed sites, where these two species are dominant. The high tolerance of water stress exhibited by *A. gilliesii* and *A. praecox* has also been reported for other species of Fabaceae from arid regions of Argentina, such as some *Prosopis* taxa (Cony & Trione 1998). The treatment with different light quality showed that all the studied *Acacia* species can germinate both under light and dark conditions. Therefore, in the dry forests of Córdoba, they are likely to germinate under the canopy of trees as well as in open gaps.

Acacia praecox and *A. gilliesii* had lower mean time to germination (i.e. they germinated faster than *A. aroma*, *A. caven* and *A. atramentaria*). This higher germination rate would be advantageous for *A. praecox* and *A. gilliesii*, allowing these species to use soil water more

efficiently under water stress conditions (Daws *et al.* 2002; Wang *et al.* 2009), a typical characteristic of the seasonally dry forests from central Argentina. On the other hand, *A. praecox* and *A. gilliesii* are dominant at closed sites, where the lowest values of soil water content were recorded.

Responses of seed germination to anthropogenic factors

Effect of simulated fire on seed germination A high frequency of fires has been reported to affect forests in central Argentina; fire is a major driver controlling the spatial distribution of closed and open habitats in the study area (Cabido *et al.* 1994; Zak & Cabido 2002). Our results show possible different effects of fire intensity (90°C 1′, 90°C 5′, 190°C 1′ and 190°C 5′) on germination of the five *Acacia* species. According to the percentages of seeds that remained hard and/or died after heat treatments, our results suggest that dormant-seeded species present at open sites (*A. aroma*, *A. caven* and *A. atramentaria*) are more capable of coping with fires of higher intensity due to their hard seed coats than nondormant-seeded species from closed sites (*A. gilliesii* and *A. praecox*; Fig. 4) (Funes & Venier 2006; Venier *et al.* 2012). Although exposure to dry heat from fires (heat shock) is considered to be an important dormancy-release mechanism, especially in *Acacia* species (Auld & O′ Connell 1991), the three *Acacia* species with physical dormancy studied here were not significantly affected by heat treatments, as their seeds were neither killed nor was their dormancy broken. Seed responses to fire in east African *Acacia* species were also found to be variable, with records of increases, decreases or no effects on germination, depending on the species (Teketay 1996). Other studies also found positive effects of fire on seed germination of Australian *Acacia* species (Auld & O′ Connell 1991).

Effect of simulated herbivore consumption on seed germination The results obtained from our simulated herbivore consumption experiment demonstrate that breaking dormancy and germination in the five *Acacia* species are likely to be differentially affected by endozoochory. For example, seeds of *A. caven* and *A. atramentaria* were not affected by the simulated ingestion by cattle, whereas *A. aroma* seeds lost physical dormancy and germinated. *Acacia aroma*, *A. caven* and *A. atramentaria* occur mainly in open habitats disturbed by livestock grazing and other factors, and there are evidences that the fruits of these species are consumed by cattle (Aronson 1992; Pensiero *et al.* 2003) and by wild herbivores in the case of *A. aroma* (Beck 2005); therefore, the regenerative stages are likely to be favored by the presence of herbivores. Fruit

consumption by cattle would have a positive effect on seed dispersion as well as on the loss of physical dormancy and germination, as in *A. aroma*. The germination percentage in *A. gilliesii* did not differ statistically from that of the control; hence, germination does not seem to be favored or highly affected by the simulated herbivore consumption. Finally, almost all of the seeds of *A. praecox* died after passing through the digestive tract of cattle; hence, we can predict a negative effect of livestock on the recruitment of *A. praecox*.

General pattern from the integration of variables

The effects of the different treatments considered separately revealed some differences in the germination patterns of the five *Acacia* species. When all the response variables explored in this study were taken together in the ordination space of the PCA, two groups of species were discriminated based on the germination characteristics: *A. aroma*, *A. caven* and *A. atramentaria* were found to be similar in germination characteristics, and therefore formed a main group, whereas, *A. praecox* and *A. gilliesii* formed another main group. Notably, the same pattern was reported in previous studies of seedlings and seed coat traits in these five *Acacia* species (Venier *et al.* 2012, 2013). According to Venier *et al.* (2012) and the environmental characteristics evaluated in the study area, the hard seed coat of *A. aroma*, *A. caven* and *A. atramentaria* would allow the seeds to endure the stressful high temperatures of open sites where these species occur. In addition, highly fluctuating temperatures of the open sites in these forests could promote loss of physical dormancy in these three species, as is reported for other species (Baskin & Baskin 2014). Specific experiments are necessary to test this hypothesis. On the other hand, the seeds of *A. gilliesii* and *A. praecox* without a hard seed coat are dispersed during the humid period of the year, when environmental conditions are favorable for germination and seedling establishment. Hence, these species do not need to prevent or delay germination after being dispersed to ensure successful seedling establishment in the xerophytic forest of Córdoba.

Based on this pattern we conclude that, although the five species seem to be very similar in vegetative traits at the adult stage (Díaz *et al.* 2004), a regenerative niche differentiation between the two groups of *Acacia* species seems to occur. Similar results are reported in the literature for other sympatric congeneric species (Vander Kloet & Hill 2000).

The general pattern obtained from the PCA ordination based on the regenerative characteristics of the *Acacia* species coincides with the regional distribution pattern of the species in the dry forests of Córdoba, Argentina: *A. aroma*, *A. caven* and *A. atramentaria* are predominantly found in disturbed open sites, whereas *A. praecox* and *A. gilliesii* occur mainly in closed forest sites (Cabido *et al.* 1994; Zak & Cabido 2002). According to part of the ecological theory, the spatial variation of environmental factors facilitates the presence of different species at diverse sites or patches as a result of habitat partition (Chase & Leibold 2003; Kneitel & Chase 2004). Thus, environmental heterogeneity can originate different spatio-temporal patterns in germination characteristics, favoring the coexistence of diverse plant species in the same geographic region (Grubb 1977; Houle 1994).

Conclusions

Our findings show that the patches of open and closed habitats that make up the vegetation mosaic of the dry Chaco forests differ in some physical factors. Our study also suggests that germination characteristics could play an important role in facilitating regional coexistence of *Acacia* species in the different types of patches occurring within the dry Chaco forests in Córdoba, central Argentina. This coexistence may be the result of dissimilar regenerative niches between the groups of species discriminated by the PCA ordination, and is likely to be in agreement with the theory of niches (Chase & Leibold 2003; Vandelook *et al.* 2008). Indeed, species that share the same type of patch are more similar in germination characteristics than those that share a different type of patch in the same region within the southern extreme of the dry Chaco. Thus, our results seem to be consistent with the ideas underlying our working hypothesis.

Acknowledgements

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Supporting information

Additional supporting information might/can be found in the supporting information tab for this article:

Appendix S1 Floristic composition recorded in each of the ten sites surveyed in seasonally dry Chaco forests from central Argentina; and number of seeds of five *Acacia* species that remained hard after the simulated fire experiment and that were then scarified and put to germinate.