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Grass competition surpasses the effect of defoliation on a woody plant invader

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ABSTRACT

Woody encroachment in grasslands represents a global phenomenon with strong consequences on ecosystem functioning. While the causes triggering this process can be multiple, there is consensus on the fact that anthropogenic activities play a central role in woody expansion. In particular, the loss of grass cover increases the chances of woody invasion, whereas the role of defoliation is less known. In this study our objective was to assess the simultaneous effect of competition generated by resident vegetation and woody seedling defoliation on the growth and survival of *Gleditsia triacanthos* seedlings, a woody invader in Argentina. We established a factorial pot experiment with two main factors: *Gleditsia* defoliation (2 levels: with and without defoliation) and pasture competition (3 levels: without pasture, clipped pasture and intact pasture). Our results showed that pasture competition reduced *Gleditsia* survival and tree growth, but that the effect of tree defoliation on tree growth depended on the magnitude of pasture competition. More widely, our results stress the existence of a hierarchy order of factors controlling *Gleditsia* establishment (survival + growth): grass competition was the main control and tree defoliation became important only in the absence of competition. This evidence suggests that maintaining a competitive grass cover along with a frequency of tree defoliation could diminish tree establishment in herbaceous communities.

1. Introduction

Woody encroachment in grasslands and savannas is a well documented phenomenon occurring around the globe (Anadón et al. 2014; Archer, 1994; Archer et al. 1988; Browning et al. 2008; Chaneton et al. 2012; Roques et al. 2001). Changes in the local and regional propagule pressure, disturbance regime and climatic variability are invoked as some of the main factors contributing to the ongoing process of woody encroachment (Archer, 1994; Bredenkamp et al. 2002; Lockwood et al. 2007; Midgley and Bond, 2001; Van Auken, 2000). The increase of woody vegetation have several consequences on community diversity (Ratajczak et al. 2012; Van Auken, 2009), ecosystem productivity (Hughes et al. 2006) and biogeochemical cycles (Archer et al. 2001; Hibbard et al. 2001; Knapp et al. 2008). Further, increase in woody cover directly affects livestock production by diminishing grass cover and also makes it difficult to maintain roads, corridors and associated agricultural environments (Anadón et al. 2014; Ghersa et al. 2002).

Several studies have demonstrated that successful woody establishment in grasslands and savannas is affected by grass competition (Grellier et al. 2012; Mazía et al. 2001, 2010; Ward and Esler, 2011) and also by seed and seedlings consumers (Archer, 1995; Grellier et al. 2012; Busch et al. 2012; Macias et al. 2014; Tjelele et al. 2015). Livestock and wild herbivores may influence tree recruitment through two opposite ways. First they may promote woody encroachment by selectively consume grasses and relaxing competitive interactions between herbaceous and woody plants (Archer, 1995; Grellier et al. 2012). Second, they may also act as a barrier to tree recruitment by consuming woody seedlings and/or by repeated mechanical damage or defoliation events (Bond, 2008; Chauchard et al. 2007; Macias et al. 2014; Riginos and Young, 2007; Roques et al. 2001).

Many studies have showed that woody plants have a variety of mechanisms of tolerance which allow them to resprout after defoliation (Haukioja and Koricheva, 2000; Strauss and Agrawal,

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1999). Tolerance to defoliation depends on the accumulated reserves which vary according to the environmental conditions (Kozlowski et al. 1991) and also with the bud bank and the foliar area retained after defoliation (Weltzin et al. 1998). Surprisingly, despite of tolerance is a key aspect in the life span of a woody plant, the ecological literature has focused more on herbaceous than on woody plants responses (Boege and Marquis, 2005; Haukioja and Koricheva, 2000). In particular, there exists scarce knowledge on how selective woody or grass defoliation may affect the process of tree establishment in grasslands and pastures.

Here we propose three conceptual models to explain the impact of woody seedlings defoliation across a gradient of grass competition (Fig. 1). In the first model the effect of defoliation and competition are additive, implying that the magnitude of the negative effect of repeated defoliation does not varies through the gradient of competition (Fig. 1a). This scenario might occur when the availability of resources is not sufficient to offset the loss of biomass, independently of the magnitude of grass competition. The second scenario presents a model of interaction where the magnitude of the negative effect of defoliation could be greater as grass competition increases (Fig. 1b). This could be the case when compensation only occurs when competition is not strong enough, at the lower extreme of the gradient. In turn, the third model suggests that the magnitude of the negative effect of repeated defoliation decreases through the gradient of competition (Fig. 1c). This could be the case when the effect of grass competition is so strong in suppressing woody seedling growth that defoliation would be irrelevant. These predictions have two important assumptions: a-competition and defoliation have both, a negative impact on the performance of a woody seedling; b-the chance of recovering after defoliation depends on the woody reserves, which will be out under the more competitive environment. In the last case, we assume a tradeoff between competitive ability and tolerance to defoliation (Coley and Barone, 1996; Maschinski and Whitham, 1989; Strauss and Agrawal, 1999).

We tested these ideas through a manipulative pot experiment using *Gleditsia triacanthos* (honey locust, hereinafter *Gleditsia*) as a model of woody plant invader. This tree is one of the most aggressive and difficult to eradicate invasive species in Argentina (Mazía et al. 2001, 2010; Ghersa et al. 2002). In previous work we showed that different factors affect the initial phase of *Gleditsia* seedling establishment in grasslands. In particular, inter-annual climatic variability (Mazía et al. 2010), disturbance regime (Chaneton et al. 2004; Mazía et al. 2010), competition from resident vegetation (Mazía et al. 2001, 2013) and seed granivory (Busch et al. 2012; Muschetto et al. 2015) in a direct or indirect way negatively affected *Gleditsia* seedling establishment (see Chaneton et al. 2012).

We examined the simultaneous effects of competition and defoliation on the establishment of *Gleditsia* seedlings. During one growing season, we analyzed the effect of defoliation on survival and growth across a gradient of grass competition generated by clipping the aerial biomass of a polyphitic pasture. Previous studies have showed that first grow season is crucial to the successful tree recruitment in pampean grasslands communities (Mazía et al. 2001, 2010). After this critical period *Gleditsia* mortality is extremely low, reflecting the survival during the initial phase of tree recruitment (Chaneton et al. 2012). Through making this experiment under controlled conditions we represented a simplified model of ecological communities which do not attempt to reproduce all natural processes occurring under field conditions. As a consequence, others factors not considered (e.g. seed dispersal, trampling as well as nutrient deposition) might also affect the probabilities of woody establishment under natural conditions (Augustine and McNaughton, 2004; Tjelele et al. 2015).

Our working hypothesis was that the magnitude of the effect of seedling defoliation varies through the gradient of grass competition. This hypothesis raises two possible predictions which are an increased or decreased defoliation effect as pasture competition increase (Fig. 1b and c respectively).

2. Materials and methods

2.1. Experimental design

We conducted a garden experiment at the Faculty of Agronomy, University of Buenos Aires (34° 35′ 32″ S; 58° 29′ 16” O). The experiment followed a factorial design with two main factors: *Gleditsia* defoliation (2 levels: with and without defoliation) and pasture competition (3 levels: without pasture, clipped pasture and intact pasture). These six treatments were disposed in 10 complete blocks. Treatments of pasture competition implied an increasing gradient of above and below pasture biomass (see below).

In September 2012, 60 pots (13.5 dm^3) were filled with fertile soil and sand (2/3—1/3, respectively) to improve drainage conditions. Twenty pots were left intact and weekly weeded (no competition) while forty were sown with a poliphytic pasture (50% of *Trifolium pratense*, 25% *Dactylis glomerata* and 25% *Bromus catharticus*). Pasture composition is the most frequently used by rangers in the Pampas. Seeds were provided by Administration de Campos (Ea. San Claudio, UBA, Carlos Casares) and tested for germination rate. To standardize the initial conditions of the experiment before *Gleditsia* planting, a first cut to all sown pots was applied to a height of 8 cm. This height corresponds to the lower limit used in rotational grazing (*Rodriguez*, personal

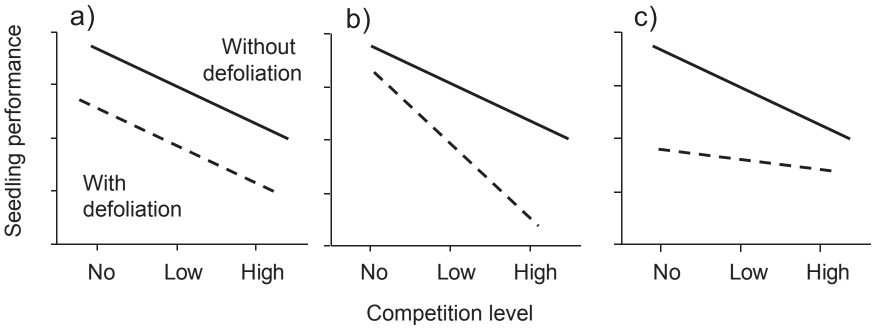

Fig. 1. Conceptual models for the performance of tree seedlings under the effect of defoliation (continuous vs. dashed lines) along an increasing gradient of competition (no competition, low competition and high competition). These models assume that both, competition and defoliation have a negative effect, but that this effect may vary. a) The negative effect of defoliation is constant under different levels of competition; there is no interaction between these factors. b) The effect of defoliation increased with the gradient of competition. c) The effect of competition surpasses the negative effect of defoliation.

communication). During the course of the experiment (November to March) all pots were watered to maintain optimal experimental conditions.

In November 2012, one *Gleditsia* seedling was planted in each pot (mean ± SE, basal diameter: 1.3 mm ± 0.04, height: 7.8 cm ± 0.24). Seedlings were produced in greenhouse by sowing scarified seeds in containers (90 cm^3). Seed scarification was produced by manual abrasion of seeds coats with sandpaper, as it produced the best results in previous assays (i.e. Mazía et al. 2013). Seedlings were maintained for a period of 5 weeks before the transplant to each pot.

The treatments of *Gleditsia* defoliation and pasture clipping were applied simultaneously every time we visualized emerging sprouts of the pasture legume *T. pratense*. This protocol was frequently used for rotational grazing among ranchers in the Pampean region (*Rodríguez*, personal communication). Following this protocol, we applied a total of 5 clip events with a frequency of ~20 days along the entire growing season (November to March). In each clipping event, cutting height (8 cm) was the same for both, pasture and tree seedlings. Defoliation was applied to *Gleditisia* plants that exceeded 8 cm height, and in general involved the removal of the top leaves and the apical bud. When *Gleditsia* plants did not reach the cutting height (8 cm) at the time of clipping pasture, they were not defoliated until the next clipping event. After each clipping event, all the removed aboveground biomass from both, pasture and *Gleditsia* seedlings were separated, dried (60 °C) and weighted. At the end of the experiment we recorded the final height and basal diameter of *Gleditsia* seedlings. Also, aerial and belowground biomass of *Gleditsia* seedlings and pasture were sorted, dried and weighted.

2.2. Data analyses

The analyses were carried out through linear mixed effects models performed in R v 2.9.0 (The R Foundation for Statistical Computing, 2009). This allowed us to model variance heterogeneity, instead of transforming the original data. First, the survival of *Gleditsia* seedlings at the end of the first growing season was analyzed with log-linear analyses, with general linear models with "logit" link in *lmer* function in "lme4" package (Crawley, 2007). The effects of competition (C), defoliation (D), and its interaction (C x D) on the surviving trees were tested with likelihood ratio chi-square statistics (Zuur et al. 2009). Second, all variables related to *Gleditsia* growth (final basal diameter, height and biomass), relative growth rate (diameter and height), shoot/root relationship (S/R) and pasture biomass, were analyzed by using two way mixed model. Relative growth rate were calculated as $\ln(x_{t1}/x_{t0})$, where x_{t1} and x_{t0} are the final and initial variable values, respectively. In these analyses, shoot biomass included all the removed biomass after each defoliation events. The model included defoliation, competition and their interaction as fixed effects and blocks as a random factor. These models were fitted with lme function ("nlme" package; Pinheiro et al., 2014). This function allows to model different variances among treatment (varIdent) instead of transforming the original data for heteroscedastic datasets. Multiple comparisons among treatments were performed with Fisher-LSD based on Walt test corrected by Bonferroni (Di Rienzo et al. 2011).

3. Results

3.1. Competition gradient

Clipping pasture declined the final aerial and root biomass by nearly 50% (Aerial: $F_{1,27} = 105.69$, $P < 0.001$; Root: $F_{1,27} = 58.04$, $P < 0.001$, Fig. 2). In contrast, *Gleditsia* defoliation had no effect on either aerial or root pasture biomass (Aerial: $F_{1,27} = 1.51$, $p = 0.229$; Root: $F_{1,27} = 1.24$, $p = 0.275$; Fig. 2). These results allowed us to confirm that the applied treatments effectively generated a gradient of pasture competition which did not vary with the level of *Gleditsia* defoliation.

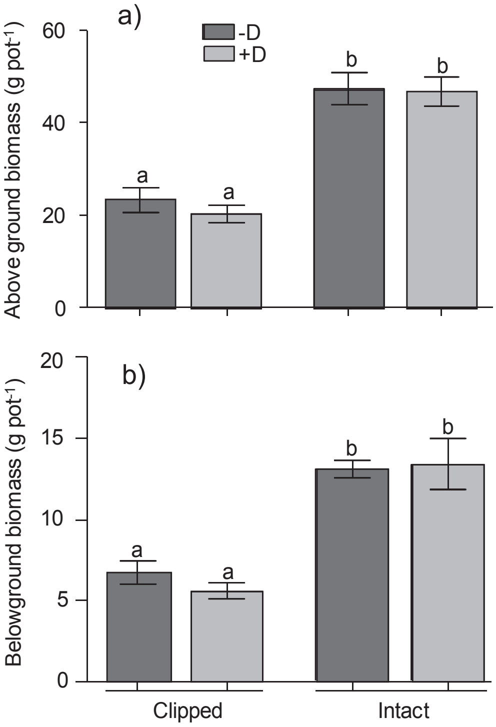

Fig. 2. Above (a) and below (b) ground pasture biomass at the end of the experiment. Each panel presents the biomass for clipped and intact pasture with (+D, light grey) or without tree seedling defoliation (-D, dark grey). Bars indicate ±1 SE. Different letters indicate significative differences $p < 0.05$.

3.2. Survival and growth

Pasture competition reduced *Gleditsia* survival independently of tree defoliation (C: $\chi^2_2 = 24.45$, $p < 0.001$; D: $\chi^2_2 = 0.07$ $p = 0.78$; C x D: $\chi^2_2 = 3.76$, $p = 0.15$). At the end of the growing season tree survival decreased from 100% through 80%–40% across the increasing gradient of pasture competition.

Both pasture competition and tree seedling defoliation significantly reduced the basal diameter, total height and total biomass of *Gleditsia* seedlings (Table 1, Fig. 3). However, the magnitude of *Gleditsia* defoliation diminished through the gradient of pasture competition (C x D: $p < 0.01$). As competition increased, defoliation reduced the basal diameter (from 48.6% through 42.42%–18.4%), plant height (from 86% through 50%–35%) and total seedling biomass (from 92% through 84%–63%, Table 1, Fig. 3). Also, the aerial *Gleditsia* biomass removed during the events of defoliation decreased across the increasing gradient of pasture competition (from 1.65g ± 0.06 through 0.37g ± 0.07 to 0.06 g ± 0.01).

The magnitude of pasture competition and tree defoliation also modified the allocation pattern of stem and root biomass (Tables 1 and 2). In particular, defoliation diminished the root biomass and the aerial biomass (from 86% through 90%–45% and from 84% through 36%–66%, respectively). As a consequence, defoliation modified the stem/root (S/R) *Gleditsia* ratio (Tables 1 and 2), the strongest effect occurred under the treatment of clipping pasture (C x D: $p < 0.001$) due to a stronger diminution of root (90%) than stem

Table 1
Results of the mixed model for the effect of grass competition and *Gleditsia* defoliation on tree growth. This table presents F ratio and *p* values for basal diameter, height, total biomass, stem biomass, root biomass and ratio stem/root biomass of *Gleditsia* seedlings at the end of the experiment. Model included blocks as random factor.

	d.f.	Basal diameter		Height		Total biomass		Stem biomass (S)		Root biomass (R)		S/R	
		F	*p*	F	*P*	F	*P*	F	*p*	F	*p*	F	*p*
Competition	2,29	577.07	<0.001	104.12	<0.001	47.62	<0.001	38.16	<0.001	61.02	<0.001	13.02	<0.001
Defoliation	1,29	302.4	<0.001	257.94	<0.001	30.40	<0.001	55.43	<0.001	41.01	<0.001	9.82	0.004
C x D	2,29	91.38	<0.001	85.80	<0.001	32.76	<0.001	28.70	<0.001	19.87	<0.001	16.66	<0.001

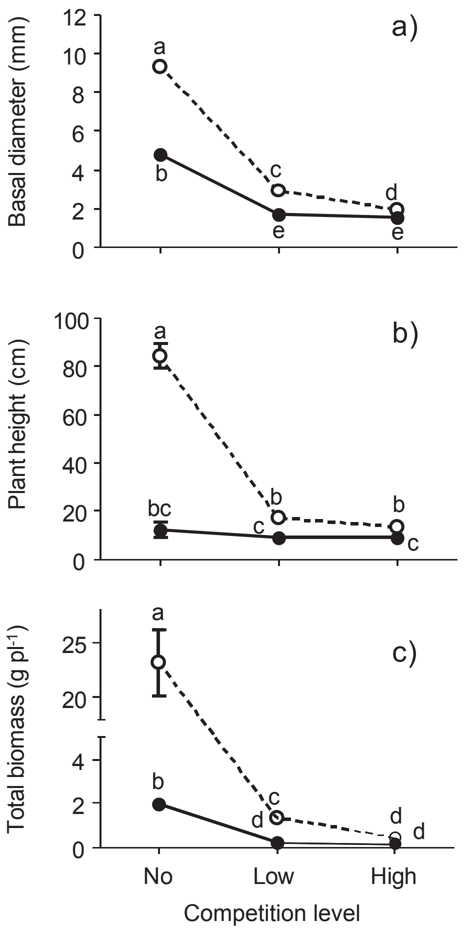

Fig. 3. *Gleditsia* seedlings mean basal diameter (a), height (b) and total biomass (c) at the end of the experiment. Intact (open symbols) and defoliated (filled symbols) woody seedlings grown in pots without pasture (no competition), with frequently clipped pasture (low competition) and intact pasture (high competition). Bars indicate ±1 SE. Different letters indicate significative differences $p < 0.05$.

biomass (36%, Table 2).

Finally, defoliation ($F_{1,29} = 37.20$, $p < 0.001$) and pasture competition ($F_{2,29} = 49.99$, $p < 0.001$) reduced the relative growth rate (RGR) in basal diameter, but this effect diminished along the gradient of grass competition (C x D: $F_{2,29} = 7.96$, $p = 0.02$, Fig. 4a). Similarly, the relative growth rate in height decreased with defoliation ($F_{1,29} = 138.82$, $p < 0.001$) and with grass competition ($F_{2,29} = 32.58$, $p = 0.001$) in different magnitude along the pasture competition gradient (C x D: $F_{2,29} = 23.81$ $p < 0.001$, Fig. 3b). Here, negative values indicate that after the last cut event *Gleditsia* did not recover its initial height.

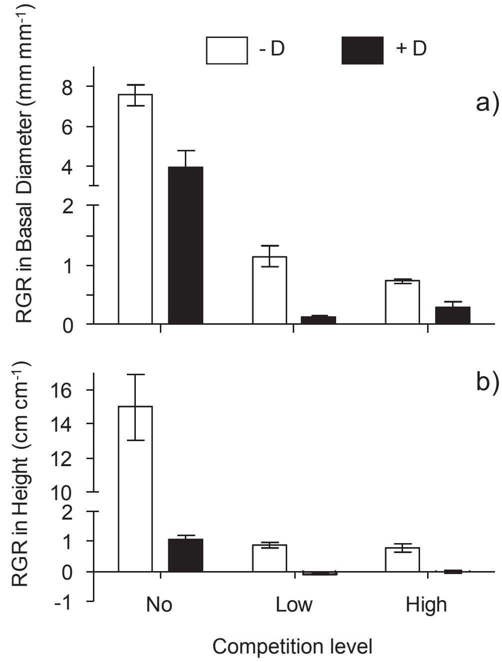

Fig. 4. Relative growth rate (RGR) in diameter (a) and in height (b) for *Gleditsia* seedlings. Intact (-D: white bars) and defoliated (+D: black bars) woody seedlings grown in pots without pasture (no competition), with frequently clipped pasture (low competition) and intact pasture (high competition). Bars indicate ±1 SE.

4. Discussion

In this study we evaluated the effects of grass competition and tree defoliation on survival and growth of the invasive tree *Gleditsia*

Table 2
Mean (±SE) biomass for tree seedlings growing under grass competition and defoliation treatments. Total biomass (g) at the end of the experiment was separated into stem and root biomass, which allowed to calculate stem/root ratio. In all cases Competition, Defoliation and C x D were significative ($p < 0.01$) after ANOVA.

Grass competition	Tree defoliation	Stem biomass (S) (g)	Root biomass (R) (g)	S/R (g g^{-1})
Without pasture	No	16.33 ± 2.16	6.75 ± 0.84	2.41 ± 0.08
	Yes	2.61 ± 0.06	0.98 ± 0.00	2.65 ± 0.06
Cutting pasture	No	0.73 ± 0.08	0.66 ± 0.07	1.35 ± 0.20
	Yes	0.47 ± 0.11	0.06 ± 0.01	12.70 ± 2.37
Intact pasture	No	0.26 ± 0.06	0.20 ± 0.05	1.38 ± 0.16
	Yes	0.09 ± 0.02	0.09 ± 0.01	2.08 ± 0.29

triacanthos. Initially, we hypothesized that the effect of tree defoliation should vary through the gradient of pasture competition. We found that woody seedling survival was only negatively affected by grass competition, whereas the magnitude of the negative effect of defoliation on seedling growth diminished when grass competition increased. These results were consistent with the model that suggested that grass competition could be more important in suppressing *Gleditsia* growth than the effect of tree defoliation (model **c** in Fig. 1).

Gleditsia survival decreased across the increasing gradient of pasture competition, while our treatment of mechanical defoliation did not modify woody seedling survival. This result suggests that grasses could have had advantages in capturing aerial and belowground resources due to its greater total biomass regarding tree seedlings biomass. This asymmetric competition might be the most common scenario at the earlier stages of a tree life, when grasses and woody seedlings share the same aerial and belowground space (Bond, 2008; Jurena and Archer, 2003). At more advanced stages of development of a woody plant, the spatial compartmentalization in the use of aerial and belowground resources might preclude tree-grass competition (Köchy and Wilson, 2000; Weltzin and McPherson, 1997). Our results are in agreement with other studies which demonstrated that plant grass cover was an effective biotic barrier to tree establishment in grasslands and savanna communities during the early life stages of woody plant invaders (De Blois et al. 2004; Dickie et al. 2007; Jurena and Archer, 2003; Mazía et al. 2001, 2013; Riginos and Young, 2007; Scholes and Archer, 1997; Ward and Esler, 2011). Here, we further showed that the magnitude of grass competition affected *Gleditsia* survival while defoliation had a secondary role. However in the long term, we cannot discard the possible negative effect of repeated defoliation events on woody seedling survival, especially during the early ontogenetic stages of a tree life when reserves to tree regrowth are scarce (Boege and Marquis, 2005; Dietze et al. 2014; Landhaeuser and Lieffers, 2012).

Pasture competition and *Gleditsia* defoliation reduced plant height, basal diameter, total biomass and the relative growth rate in height and basal diameter. Interestingly, the magnitude of the negative effect of defoliation diminished across the increasing gradient of plant competition. This was evident by the strong diminution of the total *Gleditsia* biomass removed after defoliation, which decreased one order of magnitude from control through low competition to high level of pasture competition. In agreement, the negative values of relative growth rate in height after defoliation (Fig. 4) are suggesting that *Gleditsia* never compensated the biomass loss under our defoliation regime. These results showed that *Gleditsia* tolerance to defoliation was extremely low, even without plant competition, when tree defoliation provoked a strong diminution of all measured growth variables. As a consequence of the low tolerance to defoliation, it is possible that under field conditions *Gleditsia* seedlings could spend several years under the control of consumers (Midgley and Bond, 2001). Overall, we suggest that there was a hierarchy order of the factors controlling *Gleditsia* growth, pasture competition being the main control while tree defoliation became more important without grass competition, which was evident in the no competition treatment.

The interactive effect of competition and defoliation also modified the S/R ratio of *Gleditsia* seedlings. Defoliated plants growing under the treatment of cutting pasture showed the greatest change in S/R ratio. This finding suggests that the recovery of aerial biomass loss after defoliation may conduct to a shortcoming of resources that implies a tradeoff between allocation to aerial or below ground growth (Kozlowsky, 1964, 1991; Waring 1987). This pattern of increasing S/R ratio after defoliation also occurred when pasture was maintained without cutting. But, under such conditions, it seems that pasture competition imposed a great restriction to the recovery of aerial and belowground biomass, as a consequence the S/R ratio was well below the values found in the treatment of clipping pasture (Table 2). Previous evidence has suggested that woody plant tolerance to defoliation could be associated with a greater proportion of biomass allocated to stem rather than roots (Stevens et al. 2008). However, in our experiment such response occurred along with a clear diminution of the aerial and belowground pasture biomass that possibly enhanced the chances of tree recovery after defoliation.

Nevertheless, such aerial recovery after defoliation did not occur under the context of high level of pasture competition. These results reinforce the idea that grass gaps represent safe sites to woody establishment in grasslands and savannas (Bond, 2008; Jurena and Archer, 2003; Wakeling et al. 2014). Here we also demonstrated that the stress imposed by pasture competition restricted the chances of tree recovery after applying a new stress, such as mechanical defoliation. This scenario did not occur when *Gleditsia* grew without competition, since under such condition woody plants showed a high recovery rate after defoliation. Overall, we suggest that under natural conditions plant competition and tree defoliation could play a key role in limiting woody encroachment in herbaceous communities.

In conclusion, we demonstrated that under controlled conditions early establishment of this invader tree can be interactively dependent on both, competition and defoliation. Our findings might help to design a grazing regime directed towards maintaining a competitive grass cover along with a defoliation regime that prevents tree seedling recovery.

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