Cattle grazing and herbaceous competition shape woody seedling establishment across environmental gradients in Neotropical savannas

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Abstract

Woody encroachment in savannas is a complex and global phenomenon that has negative impacts on the forage availability and livestock yield. We examined the impact of cattle grazing and herbaceous vegetation on Vachellia caven and Vachellia aroma, the main encroacher tree species in Neotropical savannas, where livestock production is the principal activity. Our experiments were conducted across a gradient of aridity and productivity (low-, medium-, and high-productivity sites), covering the central distribution of savannas in Argentina. We carried out manipulative experiments with cattle grazing (grazed–ungrazed) and herbaceous vegetation (with–without) to examine Vachellia seed loss (e.g. granivory), seedling emergence, survival, growth, and establishment. In the medium-productivity site, seed loss was higher under ungrazed conditions with herbaceous vegetation. Across all sites, cattle grazing decreased the final seedling survival regardless of the presence of the herbaceous vegetation. Herbaceous vegetation increased tree seedling survival in the medium-productivity site but decreased it in the low- and the high-productivity sites. Overall, the effect of grazing on tree establishment was neutral in the medium-productivity site as a consequence of increasing seed availability and decreasing seedling survival. However, seedling establishment was higher under ungrazed conditions and without herbaceous vegetation in the high- and low-productivity sites, because of the negative effects of grazing and herbaceous vegetation on seedling survival. From this demographic approach, we identified an ecological window during which grazing could be effectively managed to control the early stages of woody encroachment if stocking is adjusted and synchronized with tree species life history and site productivity.

Introduction

Worldwide savannas are threatened by woody encroachment processes—the increase in woody biomass, stem densities or woody cover—with negative consequences for herbaceous biomass, forage, and livestock production (Burkinshaw and Bork 2009; Van Auken 2009; Wigley et al. 2009; Eldridge et al. 2011; O'Connor et al. 2014; Stevens at al. 2017; Archer et al. 2017). Also, savanna ecosystems are home to 20% of the Earth’s population and sustain most rangelands and livestock worldwide (Scholes and Archer 1997). Therefore, the consequences of changes in tree-grass abundance represent an alteration in ecosystem functioning, with consequences for local populations that depend on ecosystem services provided by savannas. Even though drivers of tree cover and abundance, such as water, nutrient availability, and disturbance regimes, have been broadly studied, as yet there are scant systematic analyses describing the mechanisms which operate across broad environmental gradients (Scholes and Archer 1997; House et al. 2003; Jeltsch et al. 2000; Sankaran et al. 2004, 2005).

The impact of cattle grazing on woody plant recruitment in savannas is complex and determined by multiple mechanisms that may be site-specific (Cipriotti and Aguiar 2012). On the one hand, cattle may directly impact tree seed germination and seedling survival by trampling and consumption (Macías et al. 2014; Tjelele et al. 2015; Morrison et al. 2019; Mochi et al. 2022; Aranda et al. 2023). On the other hand, soil compaction, dung fertilization, and herbaceous biomass consumption have also been described as common indirect impacts of cattle grazing on tree plant recruitment (Scholes and Archer 1997; Kraaij and

Ward 2006; Grellier et al. 2012). For example, grazers can compact the soil and so reduce germination and seedling emergence (Grellier et al. 2012; Macías et al. 2014; Tjelele et al. 2015). Grazers have also been shown to facilitate tree recruitment by diminishing grass cover (Van Auken 2000; Bond 2008; Grellier et al. 2012). In this way, cattle grazing reduces tree-grass competition and fuel load, which changes the fire regime (Van Auken 2000; February et al. 2003; van Langevelde et al. 2003; Riginos and Young 2007; Bond 2008).

Tree-grass interactions in savannas have also been documented as a major determinant of savanna structure; in particular, grasses can negatively or positively affect woody plant establishment (Scholes and Archer 1997; Riginos 2009). For example, given that the presence of herbaceous cover alters the light and temperature conditions of the microsite, grass presence may reduce the germination rate of woody plants (Grellier et al. 2012). However, for several *Vachellia* species it has been demonstrated that light availability is not a limiting factor for seed germination and seedling emergence (O'Connor 1995; Funes and Venier 2006; Kulkarni et al. 2007). Grasses may also affect seedling survival and establishment. On the one hand, grasses can suppress tree seedlings through aerial and/or belowground competition for light, soil water, and nutrients (Scholes and Archer 1997; Bond 2008; Cramer et al. 2010; Cramer et al. 2012; Grellier et al. 2012; Macías et al. 2014; Pillay and Ward 2021). On the other hand, grasses can also facilitate tree establishment by sheltering seedlings from extreme temperatures and radiation (Maestre et al. 2003; Niinemets 2010; Good et al. 2014).

Several environmental factors, such as resource availability, disturbance regimes, climate, and soil type, significantly influence the interactions among trees, grasses, and herbivores in savannas (Scholes and Archer 1997; House et al. 2003; Bond and Midgley 2012; Moustakas et al. 2013; Soliveres and Maestre 2014). First, resource availability has been suggested as a factor shaping the direction and magnitude of plant–plant interactions (Bertness and Callaway 1994; Bertness and Hacker 1994; Scholes and Archer 1997; Goldberg et al. 1999; House et al. 2003; Bond 2008; Bond and Midgley 2012; Soliveres and Maestre 2014). For example, whereas in mesic savannas grasses and tree seedlings may compete for water and nutrients, in drier savannas the positive effect of the shade may outweigh the negative effects of this direct competition (Dohn et al. 2013; February et al. 2013). In savannas, rainfall also plays a crucial role in determining herbaceous productivity and, consequently, the biomass with which tree seedlings compete in the establishment phase. Second, the effect of grazing on woody populations depends on climatic conditions, as resource availability (*e.g.*, water) influences resistance and tolerance to herbivory (Hawkes and Sullivan 2001; Wise and Abrahamson 2007; Gonzales et al. 2008; Lin et al. 2021). Therefore, the impact of grazing on woody encroachment processes may vary across environmental gradients (Grellier et al. 2012). However, the influence of the environmental context on woody encroachment remains a subject of ongoing debate, partly because most studies are site-specific and conducted at small scales (Sankaran et al. 2005). In this context, understanding which of the mechanisms behind tree plant establishment are consistent across broad environmental gradients, and which are site-dependent, would enable the formulation of strategic management policies aimed at curbing the expansion of woody vegetation in savanna ecosystems.

In this study, we evaluated the effect of cattle grazing and the herbaceous vegetation on seeds and early stages of *Vachellia caven* and *Vachellia aroma* plants, two widely spread encroacher species in Neotropical savannas (Fuentes et al. 1986; Kunst et al. 2001; Van de Wouw et al. 2011; Macías et al. 2014). We evaluated the impact of cattle grazing and the herbaceous vegetation on germination, seedling emergence, survival, growth, and finally tree establishment. We focused on the early stages of tree growth as these are the most crucial stages. Once these stages have been successfully completed, tree establishment is ensured (Harper 1977; Higgins et al. 2000; Radford et al. 2001; Wiegand et al. 2006). Across aridity and productivity gradients, we tested the following hypothesis using the two species of *Vachellia* as phytometers of their savanna ecosystems:

1. Cattle grazing has a negative effect on germination and seedling emergence due to direct trampling.
2. Light availability does not limit germination and emergence rates of these species; therefore, the herbaceous vegetation does not modify tree germination and seedling emergence along the productivity gradient.
3. Cattle grazing has a negative effect on seedling survival and growth due to direct trampling and consumption.
4. The herbaceous vegetation has a negative effect on seedling survival and growth due to competition among tussock grasses and tree seedlings for water, nutrients, and/or light availability. This effect increases with an increase in productivity.

Methods

Study sites

We carried out field experiments in three savanna ecosystems in the Neotropics, Argentina (hereafter sites). The sites were situated along an aridity gradient, which is a proxy for water availability and corresponds to a productivity gradient. For each site, we estimated the aridity index (AI) using The Global Aridity Index dataset (Trabucco et al. 2008; Zomer et al. 2022; Table 1). The AI is the ratio between mean annual precipitation and potential evapotranspiration. Unlike mean annual or monthly precipitation, this index also includes atmospheric water demand and therefore provides a better approximation of hydric balance (Knapp & Smith 2001). Our study sites also present a range of aboveground herbaceous productivity, from ~ 1.5 tn ha^{-1} $year^{-1}$ dry aerial biomass to ~ 5.9 tn ha^{-1} $year^{-1}$ (Pizzio et al. 2021). We estimated the aboveground primary productivity for the years in which we conducted the experiments in our study sites by harvesting the aboveground herbaceous biomass (see *Experimental design* and Table 1). We assumed that green biomass at the end of the growing season (as estimated in this study) is equivalent to one year's growth productivity (Sala and Austin 2000; Jobbágy and Sala 2000; Oñatibia et al. 2015; Oñatibia and Aguiar 2016). The semi-arid, less productive site is in the southwest of Chaco province (S27°59', W61°25'; hereafter low-productivity site). The intermediate site in terms of AI and aerial productivity is in Entre Rios Province (S32°45', W58°28'; hereafter medium-productivity site), and the most

humid and most productive site is in Corrientes province (S29º10', W58º01'; hereafter high-productivity site) (see Supplementary Information, Fig. S1).

These three sites are dominated by *Vachellia* and *Neltuma* (formerly *Prosopis*) trees, as well as *Baccharis* and *Austroeupatorium* shrubs. Herbaceous layer composition varies among sites (Table 1), but common grass genera are *Bothriochloa*, *Nassela*, *Paspalum, Briza*, and *Schizachyrium*. Since the 19th century, the disturbance regime in all the sites has been mainly controlled by domestic cattle grazing because fires have been suppressed or controlled. The stocking rate varied among sites, with a rotational regime managed with electric or five-wire fences (Table 1). Grazing paddocks covered approximately 250–500 ha.

Woody encroachment, primarily driven by *Vachellia* species, is pervasive and occurs at all sites (Kunst et al. 2001, Macías et al. 2014, Coria et al. 2021). To carry out seedling emergence, survival, and growth experiments, in each study site we chose the most representative and abundant species that was dominant in the encroachment process: *Vachellia aroma* in the low- productivity site and *Vachellia caven* in the other two sites.

Table 1
Savanna study site characteristics.

	Low-productivity site	Medium-productivity site	High-productivity site
Site name	Chaco	Entre Rios	Corrientes
LAT	S27°59'	S32°45’	S29º10'
LONG	W61°25'	W58°28’	W58º01'
Dominant tree	*V. aroma*	*V. caven*	*V. caven*
Aridity index	0.49	0.68	0.74
Mean annual precipitation (mm)	860	1,139	1,440
Mean annual temperature (ºC)	22	17.5	19.7
Soil type	Alfisols	Vertisols and Inceptisols	Mollisols
Clay content (%)	23.78	22.3	29.4
Soil organic carbon content (‰)	1.91	4.15	2.2
Dry herbaceous biomass ($g.m^{-2}$)	235.7	500.0	522.7
Herbaceous layer	Dominated by *Sporobolus spartinus* accompanied by short C4 grasses such as *Setaria geniculata* and *Cynodon dactylon*.	Mainly C3 grasses (*Briza*, *Bromus* and *Nassella* genera among others) and dicotyledonous species.	*Andropogon lateralis* and other C4 grasses of the genera *Paspalum*, *Schizachyrium*, and *Bothriocloa*
Stocking rate ($LU.ha^{-1}$)	0.3	0.4	0.9

Experimental design

Following the same protocol, we carried out two experiments in each savanna site to evaluate the effect of cattle grazing and the herbaceous vegetation on 1) *Vachellia* seedling emergence (emergence

experiments) and 2) *Vachellia* seedling survival and growth (survival and growth experiments). For both types of experiments, at each site during the fall of 2016, at the time of fruit ripening for both species (March and April), we harvested *Vachellia* seeds from 50 adult trees. Some of the seeds were sown later in the field for emergence experiments (see *Emergence experiments*), and others were employed to grow seedlings in greenhouses (see *Survival and growth experiments* below). The seeds used in each site and in each experiment were harvested locally.

In each site, we selected four experimental sites (hereafter blocks) ~ 0.5 to 1 km apart from each other, except in the low-productivity site, where due to logistical inconvenience, we delimited three blocks instead of four. In each block, we established two 30 × 30 m experimental areas: one grazed and the other wired off to exclude grazing. We planned a two-factor split-plot design (Fig. 1). Each pair of grazed (G) vs. ungrazed (UG) areas constituted the main plots. Within the main plots (G and UG), we randomly applied herbaceous vegetation removal and established subplots with or without the herbaceous vegetation (H + and H-, respectively). In this way, we obtained a total of four treatments: G H+, G H-, UG H+, and UG H- (Fig. 1). The number of subplots with and without the herbaceous vegetation varied among experiments and sites according to the availability of seeds for carrying out the experiments (see *Emergence experiments* and *Survival and growth experiments* below). Herbaceous removal treatments (H-) were conducted manually in 30 cm diameter circular subplots where grasses and forbs were cleared using a hoe and shears and where roots were destroyed and removed, minimizing soil disturbance (Mochi et al. 2022). This procedure was applied during subsequent visits to the sites during the following growing seasons (spring 2017, summer 2017/18, spring 2018, and summer 2018/19).

Once the exclosures had been set up, we measured the aboveground herbaceous biomass in grazed and ungrazed plots two to three times over two growing seasons. Aboveground herbaceous biomass was harvested from 5 quadrants (20 cm x 50 cm) placed within each plot (grazed and ungrazed), but in a distinct surface from the subplots where the experiments were conducted. It was then dried at 60°C and weighed. On average, the aboveground herbaceous biomass in grazed conditions was lower than in ungrazed plots in the high- and medium-productivity sites ($p < 0.05$) and marginally lower in the low-productivity site ($0.05 < p < 0.1$; see Supplementary information, Fig. S2).

a) Emergence experiments

In each savanna site, in each plot (G and UG) of each block, we randomly selected subplots. The number of selected subplots per plot varied between sites, according to seed availability. We established a total of 16 subplots per plot, with a total of 512 seeds in the high-productivity site, 20 subplots per plot, with a total of 640 seeds in the medium-productivity site, and 12 subplots per plot, with a total of 360 seeds in the low-productivity site. Randomly, we removed the herbaceous vegetation in half of the subplots (H- as explained above) and left the other half intact (H+). We sowed five scarified seeds in a 90 cm^3 nursery pot in the center of each subplot. Pots were filled with sieved soil—to discard non-experimental seeds—from each subplot and buried into the ground, where they were not visible to grazers. To hasten germination seeds were scarified by manual abrasion (Ferreras and Galetto 2010; Venier et al. 2013, Mochi et al.

2022). Our experiment did not aim to evaluate natural emergence rates, but rather to estimate the effects of cattle grazing and herbaceous vegetation on this process. The experiments started at the beginning of the growing season in October 2018. Only in the medium-productivity site, this experiment was also carried out in October 2017. We collected all nursery pots 28 days after sowing and counted the number of emerged seedlings, remaining seeds without signs of germination, and lost seeds (*e.g.*, to granivores). At this time, emerged seedlings had cotyledons and one or two leaves. We assessed the seed loss rate as the proportion of lost seeds to the number of sown seeds, and the seedling emergence rate as the proportion of emerged seedlings to the number of remaining seeds (*i.e.*, sown seeds minus lost seeds).

b) Survival and growth experiments

In greenhouses, we grew *V. caven* seedlings from the high- and medium-productivity site and *V. aroma* seedlings from the low-productivity site for later transplantation to their field of origin. Seedlings were grown from scarified seeds, at the end of the winter and during springtime (see Supplementary information, Table S1). We reduced the frequency of watering and exposed seedlings to full sun for three weeks before the field transplantations. In each plot (G/UG) of each site, we placed subplots. The number of subplots per plot varied between sites according to the number of surviving seedlings in the greenhouses (see Supplementary information, Table S1). Randomly, we removed the herbaceous vegetation in half of the subplots (H- as explained above) and left the other half intact (H+). In the center of each subplot, we planted one seedling. We carried out this experiment for a total of 246 *V. aroma* seedlings (18 seedlings × four treatments × three blocks) in the low-productivity site and for a total of 400 *V. caven* seedlings (25 seedlings × four treatments × four blocks) in the medium- and high-productivity sites. For the first two to three weeks of the experiments, we replaced seedlings that died due to transplantation shock—mainly attributed to hydric stress—and obtained a complete cohort in each site (see Supplementary information, Table S1). The seedling survival and growth experiments began at that moment. At the beginning of the experiments, the seedlings had a similar size distribution for all the sites and treatments (see Supplementary information, Fig. S3). All seedlings were characterized by the presence of a tap root and a few secondary roots. For 19 months in the medium-productivity site and 18 months in the low and the high-productivity sites we monitored the transplants, by visiting each site multiple times during the growing seasons (see Supplementary information, Table S1). During each visit, we recorded seedling survival. We measured the height and basal diameter of the surviving seedlings at the end of the experiment using a ruler and a caliper, respectively.

Data analysis

All analyses were performed with the R (vs. 3.5.0) software (R Development Core Team 2019). We used generalized linear mixed models (GLMMs) assuming a binomial error distribution to estimate the effect of grazing and the herbaceous vegetation at each site on seed loos, seedling emergence, and survival rates (*lme4* package, *glmer* function; Zuur et al., 2009; Bates et al., 2015). With surviving seedlings, we calculated the annual relative growth rate in basal diameter (RGRd) and height (RGRh) as follows:

$$RGR_d = [LN(\text{diameter}_{final}) - LN(\text{diameter}_{initial})] / [t_{final} - t_{initial}] \times 365 \text{ days},$$

RGR_h = [LN (height $_{final}$) - LN (height $_{initial}$)] / [t $_{final}$- t $_{initial}$] × 365 days,

where, diameter and height were measured in mm and cm, respectively, and time in days. The resulting unit for RGR_d is (mm mm^{-1} $year^{-1}$) and for RGR_h (cm cm^{-1} $year^{-1}$) (Evans 1972; Hunt 1982, 1990; Kohi et al. 2010; Pillay and Ward 2021). We analyzed the effect of treatments on seedling growth (RGR_d, RGR_h) with mixed linear models (LMM; *lme4* package *lmer* function; Bates et al. 2015). Cattle grazing, herbaceous vegetation, site, doble and triple interactions were modeled as fixed effects, while subplots were nested within main plots, within blocks, and within site as random effects in GLMMs and LMMs. For all the models, we performed Type II ANOVA (*car* package, *Anova* function; Fox and Weisberg 2019), estimated means and confidence intervals with *lsmeans* package, *lsmeans* function (Lenth 2019) and conducted Tukey post-hoc mean comparisons. When site and any other factor interaction (doubles and triples) was statistically significant, separated post-hoc comparisons were conducted for each savanna site to better interpretation of the results. We checked for linear model assumptions (*residplot* function, *predictmeans* package; Luo et al. 2018). We also performed survival curves for each savanna site separately, by GLMMs. This approach allowed us to estimate seedling survival rate (*e.i.* proportion of surviving seedlings to the number of transplanted seedlings) during each visit to every site. We modeled cattle grazing, herbaceous vegetation, and date as fixed effects. Since we visited each plot multiple times, we nested the date within subplots, within main plots, and within blocks.

Finally, we combined the results from the emergence and survival experiments in each site to predict the overall probability of a single seed becoming established as a sapling, considering our two main factors: the presence of cattle grazing and herbaceous vegetation. The probability of establishment under the four treatments was estimated as a product of two components: 1-the probability of a seed not being lost (*e.g.*, by granivory), germinating and emerging as a seedling and 2- the probability of a seedling surviving to 18/19 months. Following the procedure from Morrison et al. (2019), we used a bootstrapping approach and resampled our data 1000 times for the model of each experiment. We estimated the probability of establishment and 95% confidence intervals from each combination of experimental treatments.

Results

Emergence experiments

The effect of cattle grazing and herbaceous vegetation on the proportion of lost seeds from nursery pots (*e.g.*, due to granivory) varied according to the study site (Table 2). In the low-productivity site, the seed loss rate was higher under ungrazed conditions with herbaceous vegetation (UG H+) than in ungrazed conditions without herbaceous vegetation (UG H-) (Fig. 2). In the medium-productivity site, the seed loss rate was higher under ungrazed conditions with herbaceous vegetation (UG H+) than under grazed conditions (Fig. 2). In the high-productivity site, we found no evidence of a significant effect of grazing or herbaceous vegetation on the seed loss rate.

The presence of herbaceous vegetation increased the *Vachellia* seedling emergence rate (emerged seedlings/remnant seeds) by 7% (Table 2), regardless of the site and grazing conditions. None of the double or triple interactions were significant (Table 2).

Survival and growth experiments

In all sites, regardless of the treatments, seedling survival decreased over time until approximately 12 months. In the low-productivity site, seedling survival was higher in ungrazed areas and marginally lower in the presence of herbaceous vegetation (Fig. 3a, Table 3). None of the interactions between the factors were significant. At the end of the experiment, no seedling survived the combined conditions of grazing and herbaceous vegetation presence. In the medium-productivity site, seedling survival was higher in ungrazed areas and in the presence of herbaceous vegetation (Fig. 3b; Table 3). At the end of the experiment, seedling survival under ungrazed conditions and with herbaceous vegetation was five times higher than in grazed areas without herbaceous vegetation (0.25 vs. 0.05). None of the interactions between the factors were significant. In the high-productivity site, the effect of herbaceous vegetation depended on cattle grazing (Fig. 3c; Table 3). Under ungrazed conditions, seedling survival was higher without herbaceous vegetation, but under grazed conditions, seedling survival was not affected by H + and H- treatments. At the end of the experiment, seedling survival under ungrazed conditions without herbaceous vegetation more than doubled the rate found in grazed conditions without herbaceous vegetation (0.63 vs. 0.24). None of the other interactions were significant.

Cattle grazing decreased the final survival of *Vachellia* seedlings across all sites regardless of the presence of herbaceous vegetation ($P < 0.05$; Table 2). The mean final survival for all the sites and herbaceous treatments was 0.273 under ungrazed conditions while it was 0.008 under grazed conditions. The effect of herbaceous vegetation on the final survival of *Vachellia* seedlings, however, depended on the site (Table 2). While in the medium-productivity site the presence of herbaceous vegetation increased final survival, in the high-productivity site it decreased final survival.

The annual relative growth rate in basal diameter (RGR_d) was found to be independent of grazing, herbaceous vegetation, and site (Table 2). In contrast, cattle grazing decreased the annual relative height growth rate (RGR_h) of *Vachellia* seedling by 61% regardless of the savanna site and herbaceous vegetation ($P < 0.05$; Table 2). None of the double or triple interactions were significant in the growth models (Table 2).

Table 2
Cattle grazing (CG) and herbaceous vegetation (H) effects on *Vachellia* seedling emergence, final survival and annual relative growth rates, among sites (S). Results from Type II ANOVA tests.

Variable	Source	$\chi 2$	*d.f*	*P*
Seed loss	**Cattle Grazing (G)**	**13.45**	**1**	**< 0.001**
	Herbaceous vegetation (H)	0.38	1	0.538
	Site (S)	**35.71**	**2**	**< 0.001**
	G×H	2.84	1	0.091
	G×S	**10.86**	**2**	**0.011**
	H×S	1.87	2	0.394
	G×H×S	**4.74**	**2**	**0.039**
Seedling emergence	Cattle Grazing (G)	2.01	1	0.156
	Herbaceous vegetation (H)	**4.31**	**1**	**0.038**
	Site (S)	2.28	2	0.320
	G×H	0.11	1	0.740
	G×S	3.26	2	0.196
	H×S	1.61	2	0.447
	G×H×S	0.82	2	0.613
Seedling final survival	**Cattle Grazing (G)**	**18.21**	**1**	**< 0.001**
	Herbaceous vegetation (H)	0.70	1	0.404
	Site (S)	**6.99**	**2**	**0.033**
	G×H	2.33	1	0.127
	G×S	1.30	2	0.633
	H×S	**9.22**	**2**	**0.010**
	G×H×S	1.80	2	0.417
RGRd ($mm.mm^{-1}.año^{-1}$)	Cattle Grazing (G)	0.87	1	0.267
	Herbaceous vegetation (H)	2.60	1	0.103
	Site (S)	2.24	2	0.619
	G×H	2.09	1	0.143

Variable	Source	$\chi 2$	*d.f*	*P*
Seed loss	**Cattle Grazing (G)**	**13.45**	**1**	**< 0.001**
	CG×S	0.21	1	0.718
	H×S	0.20	2	0.905
	G×H×S	0.05	1	0.831
RGRh ($mm.mm^{-1}.año^{-1}$)	**Cattle Grazing (G)**	**12.36**	**1**	**< 0.001**
	Herbaceous vegetation (H)	0.39	1	0.533
	Site (S)	0.06	2	0.953
	G×H	0.22	1	0.639
	G×S	0.01	1	0.972
	H×S	4.95	2	0.084
	G×H×S	0.38	1	0.538

Results indicate Chi^2 and *P* values for type II test for the fixed factor effects on seed loss, seedling emergence, survival, relative growth rate in basal diameter and relative height growth rate. *P* values in bold are significant (< 0.05)

Table 3
Cattle grazing (G), herbaceous vegetation (H) and date (D) effects on *Vachellia* seedling survival for each site. Results from Type II ANOVA tests.

Source	Low-productivity site			Medium-productivity site			High-productivity site		
	$\chi 2$	*d.f*	*P*	$\chi 2$	*d.f*	*P*	$\chi 2$	*d.f*	*P*
Cattle Grazing (G)	7.16	1	**0.007**	5.28	1	**0.022**	8.35	1	**0.003**
Herbaceous veget. (H)	3.67	1	**0.055**	28.46	1	**< 0.001**	20.04	1	**< 0.001**
Date (D)	11.72	2	**0.004**	234.41	4	**< 0.001**	14.69	2	**< 0.001**
G*H	0.03	1	0.873	0.29	1	0.591	7.51	1	**< 0.001**
G*D	2.34	2	0.310	2.92	4	0.572	4.13	2	0.127
H*D	0.05	2	0.975	2.42	4	0.659	1.12	2	0.570
G*H*D	0.09	2	0.952	0.75	4	0.945	1.35	2	0.510

Results indicate Chi^2 and *P* values for type II test for the fixed factor effects on seedling over time for each site. *P* values in bold are significant (< 0.05)

Under our four experimental conditions, at each savanna site, we also estimated *Vachellia* establishment rates, as the probability of a seed not to be lost (*e.g.*, by granivory), to germinate and to emerge as a seedling and survive to the sapling stage (19th month). In the medium-productivity site we found no evidence that *V. caven* probability of establishment varies with cattle grazing and/or herbaceous vegetation, since the confidence intervals overlapped with the means of all treatments (Fig. 4b). In contrast, in the low- and high-productivity sites, *Vachellia* probability of establishment under ungrazed conditions and without herbaceous vegetation (UG H-) was higher than under grazing (GH- and GH+), but not different from the ungrazed conditions with herbaceous vegetation (Fig. 4a and 4c).

Discussion

On a productivity gradient, we examined the effect of cattle grazing and herbaceous vegetation on different demographic transitions that may act as bottlenecks during the early stages of savanna woody encroachment (Sankaran et al. 2004). Our results showed that along the productivity gradient, the treatments had different effects on shaping the final rate of woody seedling establishment. However, each variable showed a different response depending on the tree life stage analyzed, from seed to seedling emergence and seedling survival.

The rate of seed loss (*e.g.*, by granivory) depended on the study site, along with the applied treatments (Fig. 2). Cattle grazing has direct and indirect effects on the granivore community (Milchunas et al. 1988, Schmidt et al. 2005, Teman et al. 2021). On the one hand, cattle grazing may directly interfere with granivores (Voeten and Prins 1999). On the other hand, cattle grazing, by changing the vegetation structure, may also indirectly impact granivore communities. In agreement, in the medium productivity site, through reduction of aerial biomass, cattle grazing could have decreased refuge availability for rodents, which in turn decreased seed consumption in comparison to the treatment under ungrazed conditions with the presence of herbaceous vegetation (Schmidt et al. 2005; Young et al. 2018; Teman et al. 2021). However, our results indicate that the effect of exclosures on granivory would be site-dependent and would depend not only on environmental characteristics, but also on the species and granivore guild identity in each environment.

Across all sites, the seedling emergence rate (*i.e.*, emerged seedlings/remnant seeds) increased with herbaceous vegetation and was not affected by cattle grazing. This result agrees with observations in other woody species, where germination and emergence rates were enhanced in patches with vegetation due to increased moisture retention compared to patches without vegetation (Borchert et al. 1989; O'Connor 1995). Moreover, previous studies have demonstrated that the quantity and quality of light do not act as abiotic constraints on the germination and emergence of several *Vachellia* and *Neltuma* species (Brown and Archer 1989, 1999; O'Connor 1995; Funes and Venier 2006; Kulkarni et al. 2007). In this regard, grasses may not interfere with Vachellia emergence through competition for light; instead, they may have created a moist microsite that enhanced seed germination and seedling emergence.

The annual relative height growth rate (RGRh) of *Vachellia* seedlings was lower under grazed conditions, irrespective of the site or the presence of herbaceous vegetation. These results indicate that cattle actively consume *Vachellia* seedlings of both species, rather than incidentally. If incidental consumption or associational susceptibility had been the case, we would have expected to find that seedlings in subplots without herbaceous vegetation under grazed conditions were consumed less than those in subplots with herbaceous vegetation (Tahvanaine and Root 1972; Karban 1997). However, this pattern was not observed at any site. In agreement, several studies have shown that seedling consumption diminishes tree seedling survival and establishment (Riginos and Young 2007; Macias et al. 2014; Morrison et al. 2019; Aranda et al. 2023). Our results reinforce the idea that grazing exerts a direct negative effect on tree seedlings, regardless of site conditions, and indicate that tree seedlings were actively included within the cattle bite. Overall, this result highlights the importance of studying the process of woody encroachment along the different life stages of a woody plant, as the “vulnerability window" may change along the tree life cycle.

The effect of herbaceous vegetation on the survival of Vachellia seedlings varied among the sites. The sign of this effect ranged from a positive effect of herbaceous vegetation on seedling survival in the medium-productivity site to negative effects in the low- and high-productivity sites. These differences among sites could be mainly explained by differences in C3-C4 dominance at different sites (see Table 1). The C3 grass layer enhanced survival at the medium-productivity site, while the C4 grass layer decreased tree seedling survival at the other sites. Several studies have demonstrated that as productivity increases, competitive relationships tend to be reinforced (Bertness and Callaway 1994; Bertness and Hacker 1994; Scholes and Archer 1997; Bond and Midgley 2012; Soliveres and Maestre 2014). Our results, however, suggest that tree:grass competition could decrease depending on the composition of the herbaceous vegetation and its photosynthetic pattern (*i.e.*, C3 vs. C4), rather than productivity itself. In this regard, C4 grasses may outcompete tree seedlings for water because of their higher water-use efficiency, greater productivity at elevated temperatures, reduced nitrogen requirements, and significant allocation of carbon to their roots (Knapp and Medina 1999). Surprisingly, this aspect has received limited attention in the literature, perhaps because there are few experimental studies such as ours that combine sites dominated by both C3 and C4 grasses. Therefore, further studies are needed to understand how species identity, growth seasonality, and the photosynthetic pathway of herbaceous plants may influence tree-grass interactions in savanna ecosystems.

Establishment is the result of different demographic transitions, each with different susceptibilities to grazing and herbaceous cover, which in turn varies along the productivity gradient. *V. aroma* and *V. caven* establishment in the high- and the low-productivity sites dominated by C4 grasses, was higher under ungrazed condition and without herbaceous vegetation (Fig. 4a and 4c). These results are explained by the negative effect of grazing and herbaceous vegetation on seedling survival, as the number of lost seeds and emergence rate did not vary between treatments for the high-productivity site (Figs. 2 and 3). In the case of the low-productivity site, the negative effect of grazing and herbaceous vegetation on seedling survival was reinforced by the positive effect of UG H + treatment on seed loss, which decreased the probability of seedling establishment. In this regard, our results showed that cattle management plays

a key role in limiting the establishment of woody seedlings and, consequently, in controlling *Vachellia* encroachment processes from the early stages. For instance, low grass cover and bare soil in the absence of cattle grazing significantly increase the chances of tree establishment. In contrast, in the middle-productivity site, the *V. caven* establishment rate did not vary between treatments (Fig. 4b). The neutral effect of cattle grazing may be explained by contrasting effects. On the one hand, grazing decreased the number of lost seeds in the emergence experiment, which determined an increase in seed availability. On the other hand, cattle grazing decreased seedling survival (Figs. 2 and 3).

Based on our results, we suggest that the management of cattle grazing pressure could be a viable strategy for controlling the early stages of the woody encroachment process in the region. On one hand, many studies have demonstrated the effect of overgrazing as a trigger of woody encroachment processes (Adámoli et al. 1990, Archer 1995, Van Auken 2000, O'Connor et al. 2014, LaMalfa et al. 2021). Bare soil would be the main determinant of this process, as many woody species identified as encroachers have been described as colonizers (Venier et al. 2013). In contrast, the exclusion of grazing has not proven to be an effective tool against woody encroachment process in the region (Macías et al. 2014; Rolhauser and Batista 2014; Batista et al. 2018). In particular, grazing stocks should be managed to maintain high herbaceous biomass and maximize tree-grass competition (*e.g.*, as in the low- and high-productivity sites). Also, cattle stocking rates should be relaxed during the primary dispersal stage of trees (late summer/autumn), because cattle could act as seed dispersers. For example, for *V. aroma*, the passage of seeds through the digestive tract has been shown to positively influence the loss of physical dormancy and germination (Venier et al. 2017). Finally, cattle stocking rates should be increased immediately after seedling emergence in spring to increase the likelihood of encounters between herbivores and tree seedlings. To do this, it would be necessary to assess what stocking rate would increase mortality at each site, without compromising the regrowth capacity of the grass layer.

Declarations

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

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Author Contribution

L.S.M., N.M and M.R.A. planned the research and designed field experiments, L.S.M, M.J.A and N.M conducted the field experiments and samplings, L.S.M. and F.B performed statistical analyses. L.S.M led the writing and all authors critically revised the manuscript.

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Figures

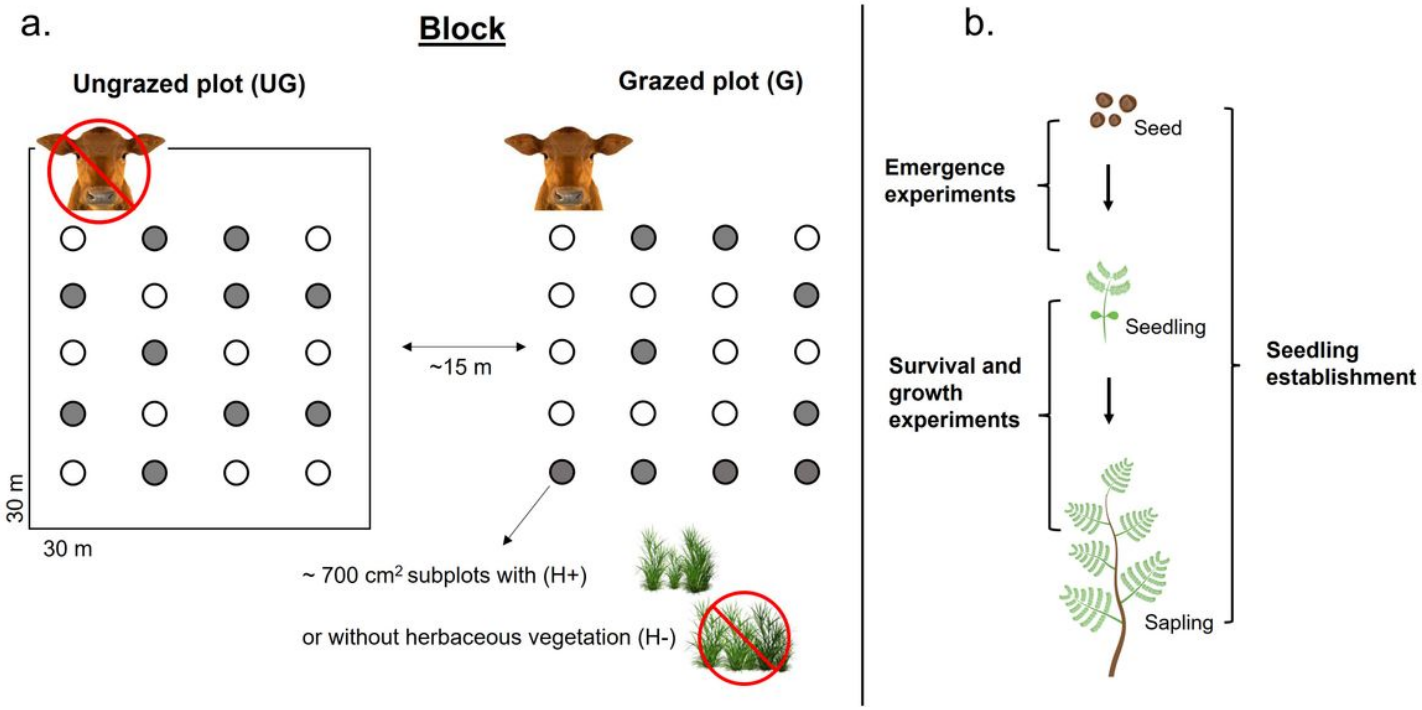

Figure 1

(a) Experimental split-plot design at each savanna site. Three blocks were selected in the low-productivity site, and four blocks were selected in the medium- and high-productivity sites. An exclosure was established in each block. Each exclosure constituted one of the main plots, and an adjacent area on grazed conditions the other main plot of each block cattle grazing treatments (grazed vs. ungrazed). Subplots with and without herbaceous vegetation are represented by grey and white, respectively (H+ and H-). The number of subplots with and without herbaceous vegetation varied across experiments and sites based on seed and seedling availability for conducting the experiments (see *Materials and Methods* for details). **(b)** *Vachellia* life stages and transitions included in our experiments.

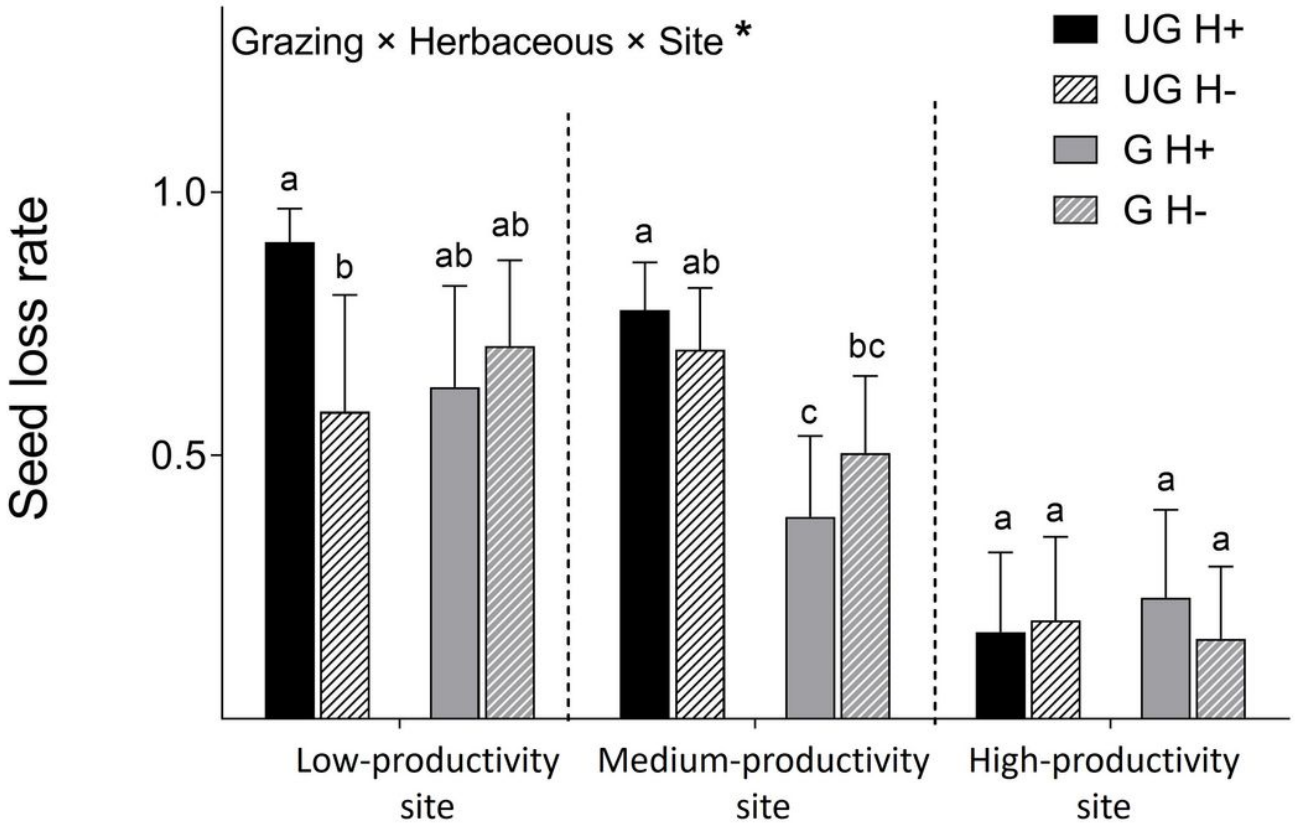

Figure 2

Seed loss rate in all sites for all treatments: ungrazed with herbaceous vegetation (UG H+), ungrazed without herbaceous vegetation (UG H-), grazed with herbaceous vegetation (G H+), grazed without herbaceous vegetation (G H-). Seed loss rate shows the proportion of lost seeds to the total number of sown seeds. Error bars indicate 95% confidence intervals. Asterisks indicate a significant interaction among the main factors (Grazing, Herbaceous vegetation, and Site). Different letters indicate significant differences ($P < 0.05$) among Grazing × Herbaceous treatments on seed loss rate at each site separately.

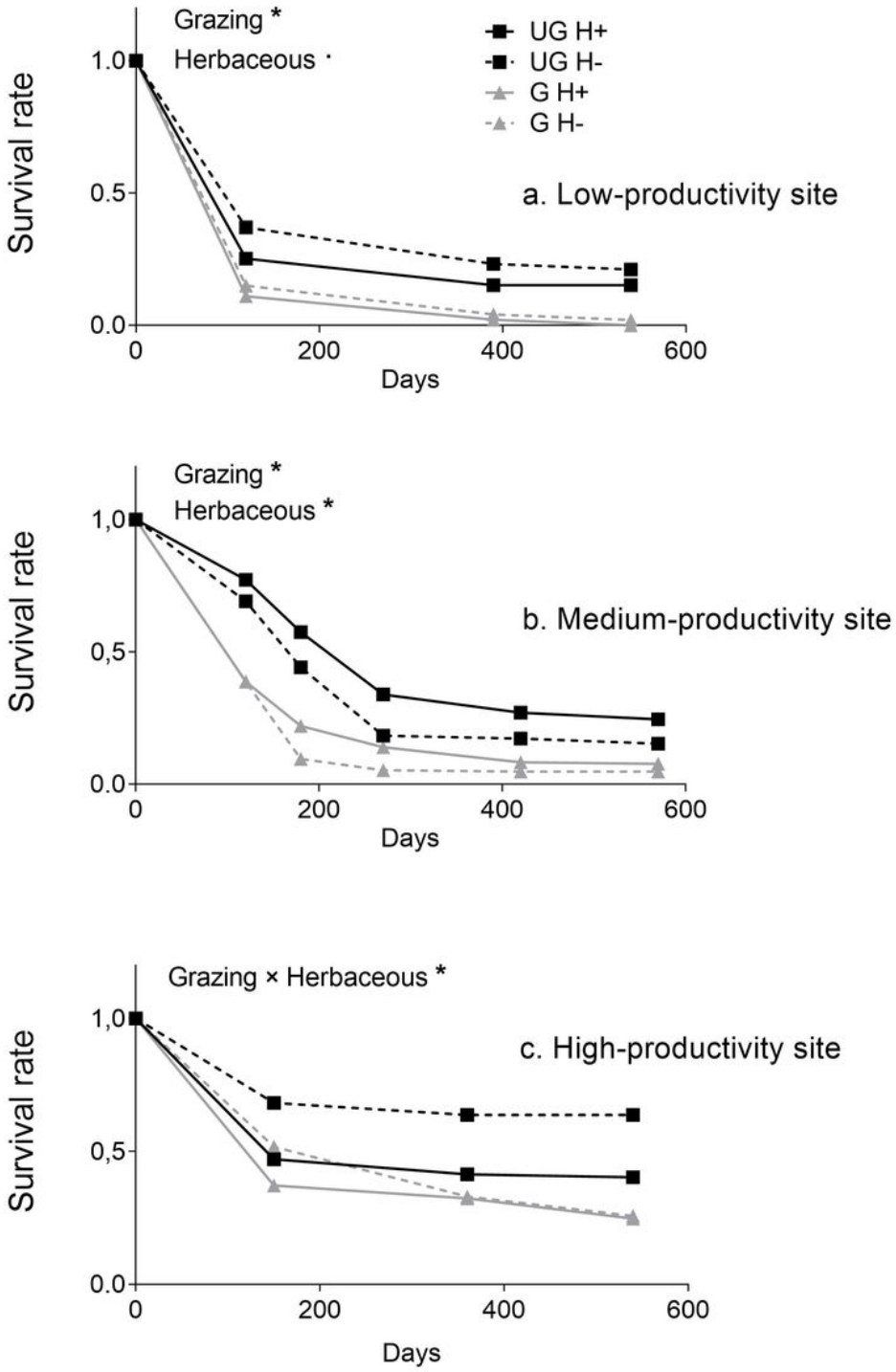

Figure 3

Survival curves of *Vachellia*seedlings for all treatments: ungrazed (black) and grazed (grey), with (H +) and without (H-) herbaceous vegetation (solid and dashed lines, respectively) over 540 days in the low-productivity site **(a)**, 570 days in the medium-productivity site **(b)**, and 540 days in the high-productivity site **(c)**. Squares and triangles represent seedling survival at each date in relation to the initial total. Asterisks indicate significant effects of grazing, herbaceous vegetation, or their interactions at a

significance level of 0.05, while the dot indicates marginal effects (0.05 < *P* < 0.1). In all sites, survival decreased over time, irrespective of the other factors.

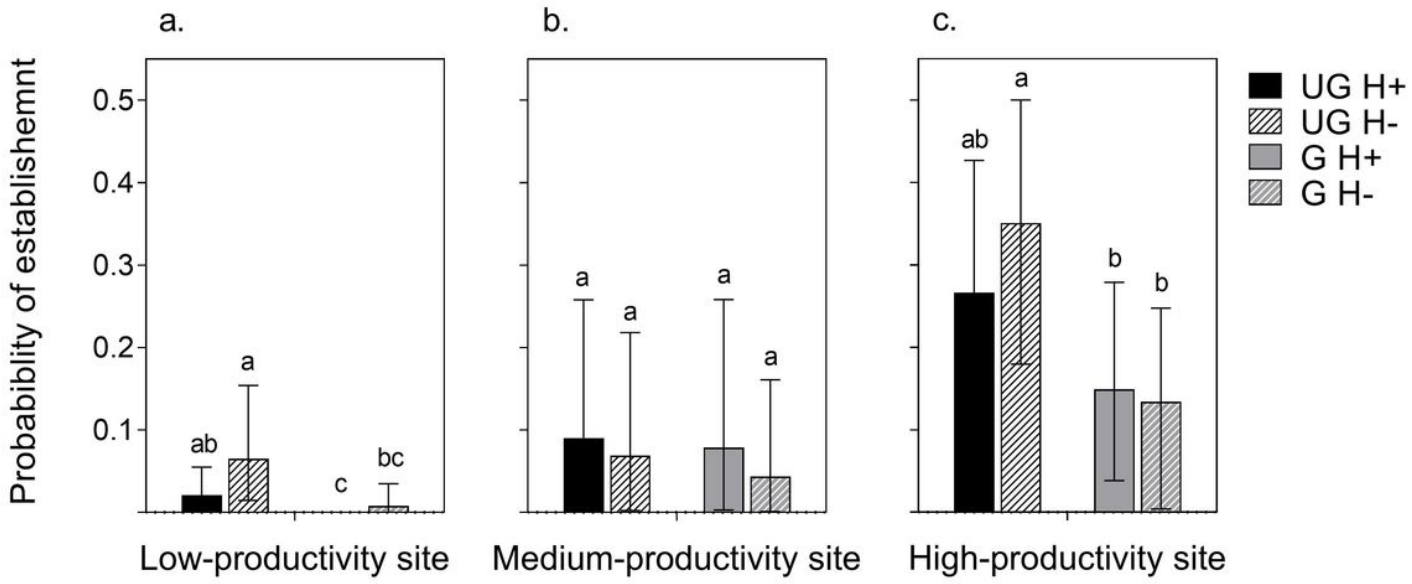

Figure 4

Predicted *Vachellia* establishment (seed germination, emergence, and seedling survival from 0 to 18-19 months) for each experimental condition (ungrazed with herbaceous vegetation (UG H+), ungrazed without herbaceous vegetation (UG H-), grazed with herbaceous vegetation (G H+), grazed without herbaceous vegetation (G H-)) at each site. Error bars indicate 95% confidence intervals. Different letters indicate significant differences among treatments for each site (*P* < 0.05). A comparison of predicted *Vachellia*establishment between sites was not possible since the bootstrapping approach was performed separately for each site. In the low-productivity site, the mean probability of establishment for the grazed with herbaceous vegetation treatment (G H+) was equal zero, as there were no living seedlings at the end of the experiment for that treatment.

Supplementary Files

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