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RESEARCH ARTICLE

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Cattle and nurse trees shape subtropical forest–grassland ecotones

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Abstract

1. South American subtropical landscapes are dominated by open grasslands and mosaics of forest–grassland formations. Forests are often restricted to riverine margins with sharp forest–grassland ecotones. Understanding the mechanisms maintaining forest–grassland ecotones is important to anticipate the effects of changing climate and disturbance regimes on the extent of these biomes and the ecosystem services they provide.
2. We used a combination of field surveys and long-term field experiments to explore the mechanisms that explain tree cover expansion at the ecotone of riverine forests and grasslands in central Uruguay, within the South American Campos. We assessed the role of tree seed dispersal and seedling establishment limitations, and experimentally tested for the effects of cattle, nurse tree cover and grasses on the recruitment of forest and grassland tree species at the forest–grassland ecotone.
3. We found that forest expansion depends on the interplay between cattle and nurse trees. *Vachellia caven* trees colonize the grassland successfully and facilitate the formation of forest patches by enhancing seed accumulation and seedling establishment of forest tree species. Surprisingly, grass cover had mostly positive effects on early seedling survival of forest tree seedlings. However, cattle limits tree seedling growth and survival, especially of forest tree species. This results in a nucleated vegetation pattern of tree patches that ultimately limits forest expansion.
4. *Synthesis and applications*. Tree cover can potentially expand on the subtropical South American grasslands. Reductions in cattle densities and increases in rainfall levels associated with climate change could facilitate forest expansion in this region.

KEYWORDS
alternative ecosystem states, facilitation, grassy biomes, herbivore–plant interactions, South America, tree recruitment, vegetation shift, woody encroachment

1 | INTRODUCTION

South American subtropical grasslands expand between tropical rainforests to the north and temperate grasslands to the south, in a biogeographic region known as the Rio de la Plata Grasslands (Soriano et al., 1991). It is a heterogeneous region, with extensive grasslands and mosaics of forest–grassland formations, which expands from central-eastern Argentina (Pampas subregion) across all of Uruguay and southern Brazil (the latter two forming the Campos subregion) surrounding the Rio de La Plata river and its major tributaries (Overbeck et al., 2023). These almost treeless landscapes have fascinated ecologists since Darwin's observations highlighted the remarkable absence of trees in a subtropical region with relatively high levels of precipitation (Darwin, 1839). In the Campos subregion, forest cover is strongly associated with riverine edges (Bernardi, Holmgren, et al., 2016) and has remained fairly constant during at least the last 200 years (Gautreau, 2010). These vegetation patterns may result from the combined legacy effects of past climates, when trees found moist and warm refuges along watercourses and deep valleys, and current disturbance regimes, preventing forest expansion into open grasslands (Behling et al., 2023; Müller et al., 2012). High livestock density across the South American Campos has been identified as a major driver keeping lower levels of tree cover, and maintaining also lower fire occurrence, than those found in climatically comparable regions across the tropics and subtropics (Bernardi, Staal, et al., 2019). Indeed, increases in tree regeneration and forest cover have been documented where livestock have been excluded (Brazeiro et al., 2018; Etchebarne & Brazeiro, 2016) or their densities reduced (Bernardi, Buddeberg, et al., 2019). Under low cattle densities, forest–grassland ecotones become shrubbier as an early indicator of woody plant expansion (Etchebarne-Palla et al., 2022).

Understanding the process of tree cover expansion in this South American biogeographic region is important for management and conservation of ecosystem services associated with both natural grasslands and forests. Grasslands provide habitat to unique plant and animal species, are reservoirs of soil carbon, contribute to soil and water retention and sustain one of the largest extensive livestock production systems worldwide (Modernel et al., 2016). On the other hand, native forests buffer run-off and input of sediment and nutrient into aquatic systems (Lucas et al., 2022), store carbon above-ground, and provide habitat connectivity that contributes to biodiversity conservation at regional and continental scales (Nores et al., 2005; Oliveira-Filho et al., 2015; Ramirez & Säumel, 2022).

Several lines of evidence suggest that across the tropics and subtropics, forests, savannas and grasslands can be alternative states of tree cover maintained by positive feedbacks (Hirota et al., 2011; Staver et al., 2011). The probability of forest occurrence increases with mean annual precipitation and shorter dry seasons, but disturbance regimes can maintain lower levels of tree cover than those expected by climate conditions only (Staal et al., 2018). Locally, forest expansion on grasslands starts as a process of shrub and tree cover expansion, often at the ecotone between both systems, where fire or grazing disturbance regimes have become less intense (Carlucci et al., 2011; Oliveira & Pillar, 2004). This process has been coined woody plant encroachment to highlight the replacement of herbaceous plants by woody shrubs and trees. Forest expansion on grasslands depends on the interplay of biotic processes, environmental conditions and disturbance regimes affecting the likelihood of trees to successfully establish and persist in the grassland. The recruiting success of new trees depends on two main processes: seed dispersal and seedling establishment. Because these two processes in the early life phases of plants are very sensitive to environmental conditions, they can limit tree recruitment by either seed limitation or establishment limitation. Seed limitation is the reduction of tree recruitment caused by the lack of seeds or the agents that facilitate their dispersal (Turnbull et al., 2000), whereas establishment limitation is the reduction in recruitment caused by the mortality of tree seedlings and saplings (Nathan & Muller-Landau, 2000). The relative importance of these different stages of tree recruitment varies depending on local contexts such as habitat connectivity and environmental conditions. In the Brazilian grasslands, on the northern range of the Campos subregion, seed dispersal and seedling establishment of forest tree species are both limiting processes facilitated by nurse trees (Duarte et al., 2006).

Livestock browsing and competition with grasses can strongly limit tree seedling establishment in subtropical grassy biomes (Macias et al., 2014). These effects can be modified by the presence of adult trees as they can influence the behaviour of herbivores (Smit et al., 2006), the abundance and composition of grasses (Bernardi, de Jonge, & Holmgren, 2016; Cayssials & Rodríguez, 2013), and also the microclimate and soil resources available for tree seedlings under their canopies (Holmgren et al., 2012). In turn, these ecological interactions depend on the overall environmental conditions as the ameliorating effects of tree shade become more important during dry and warm conditions for herbivores (Scharf et al., 2010), grasses (Hernández-Salmerón & Holmgren, 2022) and young tree seedlings (Holmgren et al., 2012). Moreover, the relative importance of biotic and abiotic factors on tree seedling establishment depends on an array of plant functional traits defining the species capacity to tolerate and recover from stressors and perturbations. For example, tough or spiny leaves can reduce palatability (Cingolani et al., 2005; Skarpe & Hester, 2008), whereas early resprouting can increase tree seedling recovery after browsing (Archibald et al., 2021). As rainfall levels increase, the role of fire becomes more important than herbivore browsing as main disturbance controlling tree cover expansion in tropical and subtropical grasslands (Staal et al., 2018). Resprouting capacity has also been identified as an important tree trait in forest–grassland ecotones maintained by recurrent fires (Müller et al., 2007). Overall, the interplay of climate, disturbances, biotic interactions and environmental heterogeneity influence the rates of forest expansion and the persistency of forest–grassland mosaics at the landscape level (Blanco et al., 2014; Müller et al., 2023) and the diversity of patterns found on tree expansion across grassy biomes (Archer et al., 2017).

Our understanding of forest–grassland dynamics in the South American Campos has been enriched by research conducted at different spatial and temporal scales. The mechanisms explaining current forest–grassland ecotones are better understood for southern Brazilian grasslands, in the moister and warmer range of the Rio de la Plata grasslands (Müller et al., 2023). Although Uruguay represents 80% of the Campos, forest–grassland ecotones have only been described for eastern hillsides (Brazeiro et al., 2018; Etchebarne-Palla et al., 2022). To better understand the mechanisms that explain tree cover expansion in this region, we used a combination of field surveys and long-term experiments at the ecotone between grasslands and riverine forests in the central plains of the Uruguayan Campos. We hypothesised that forest expansion in grasslands is facilitated by isolated nurse trees and limited by cattle browsing and grass competition. We predicted that forest tree species would recruit more successfully under nurse trees than in open grassland as seed dispersal and seedling establishment would be enhanced under the nurse tree canopy. We also expected that the relative abundance of forest tree species would increase as tree patches increase in size. We conducted a field experiment to assess the effects of cattle, grasses and nurse trees on seedling emergence and survival of tree species associated with either forests or grasslands. To assess whether the relative abundance of forest tree species increases as tree patches increase in size, we assessed seed dispersal, tree structure and species composition along a gradient of tree patch sizes naturally established on grasslands.

2 | MATERIALS AND METHODS

2.1 | Study system

The study site was located in central Uruguay, 10 km from Durazno within a 300-ha rangeland property (33°23′ S, 56°31′ W) (Figure 1). Mean Annual Precipitation is 1300 mm. Mean Monthly Temperatures fluctuate between 23.8°C (January) and 11.8°C (July) (Berretta et al., 2000). Soils are rich in organic matter and have good drainage, classified as Brunosol Subeutric (Álvarez et al., 2015). Forests are restricted to the margins of the river Yi forming a dense band up to 1250 m wide. Between the forest edge and the open grassland, tree cover expands as a mosaic of dense tree patches and scattered trees until only few solitary trees are found in the treeless grassland (Figure 1). *Vachellia caven* (Fabaceae, ex. *Acacia caven*) is the most abundant solitary tree species growing in the grassland. The open grassland is dominated by C4 grasses, whereas C3 grasses are more abundant under the canopy of solitary trees (Bernardi, de Jonge, & Holmgren, 2016). These grasslands support extensive cattle production (0.6–0.8 ind/ha) of mostly Angus and Hereford breeds (Figure 1). The landowner and manager authorized the use of this property to conduct the study. We further did not require ethics approval or fieldwork permits.

2.2 | Patterns of tree cover expansion

2.2.1 | Tree patches: Size, distribution, structure and species composition

We used aerial images available in Google Earth and Image J software to measure the size and distance from the river of 1744 tree patches scattered within an area of 1125 by 425 m of forest–grassland ecotone (Figure 2a).

To assess tree size distribution and species composition, we randomly selected 12 patches of each of the following tree patch size categories (based on canopy diameter): solitary trees (<4 m, no neighbouring trees), small patches (<8 m), medium patches (8–16 m) and big patches (16–36 m) (Table S1.1). In the field, we measured the two perpendicular canopy diameters of each tree patch and used their mean to calculate patch area as $A = \pi \times r^2$, thus characterizing the range from solitary trees ($12.68 \pm 1.66\,\text{m}^2$) to small patches ($34.18 \pm 4.50\,\text{m}^2$), medium patches ($100.\ 32 \pm 11.82\,\text{m}^2$) and big patches ($347.77 \pm 60.68\,\text{m}^2$), where patches were always formed by several individual trees of more than one species. Within each tree patch, we counted and identified all juveniles (<1 m high), adults and dead trees. We also recorded height and diameter (DBH) of the tallest and thickest tree growing in the tree patch.

2.2.2 | Tree functional traits

To explore the role of plant functional traits on successful tree recruitment, we assessed specific leaf area (SLA; cm^2/g), leaf toughness and tree spininess on adult trees. These traits are associated with interspecific differences in growth rate, and tolerance to water stress and grazing (Cingolani et al., 2005; Saatkamp et al., 2010). We took a composite sample of three young full-grown apical leaves from the tallest adult of each tree species present in every patch. We used the protocols described by Perez-Harguindeguy et al. (2013) to collect and process the samples. Overall, we collected 879 leaves from 293 adult trees of 17 species. We calculated the community trait-weighted mean for SLA and leaf toughness by multiplying the mean of the three leaf measurements per trait, taken from adults of each tree species, by the number of adult individuals of the species found in the patch. Finally, we calculated the overall sum across all species and divided this by the total number of adults in the patch, resulting in an average tree leaf trait for every patch. Tree spinescence was measured using a binary scale (presence/absence) to calculate the proportion of adult trees with spines present in a patch.

2.2.3 | Seed rain and seed bank: Abundance and composition

We assessed the seed rain and soil seedbank of grassland, the four tree patch size categories and forest ($n = 6$ for each habitat type). Within

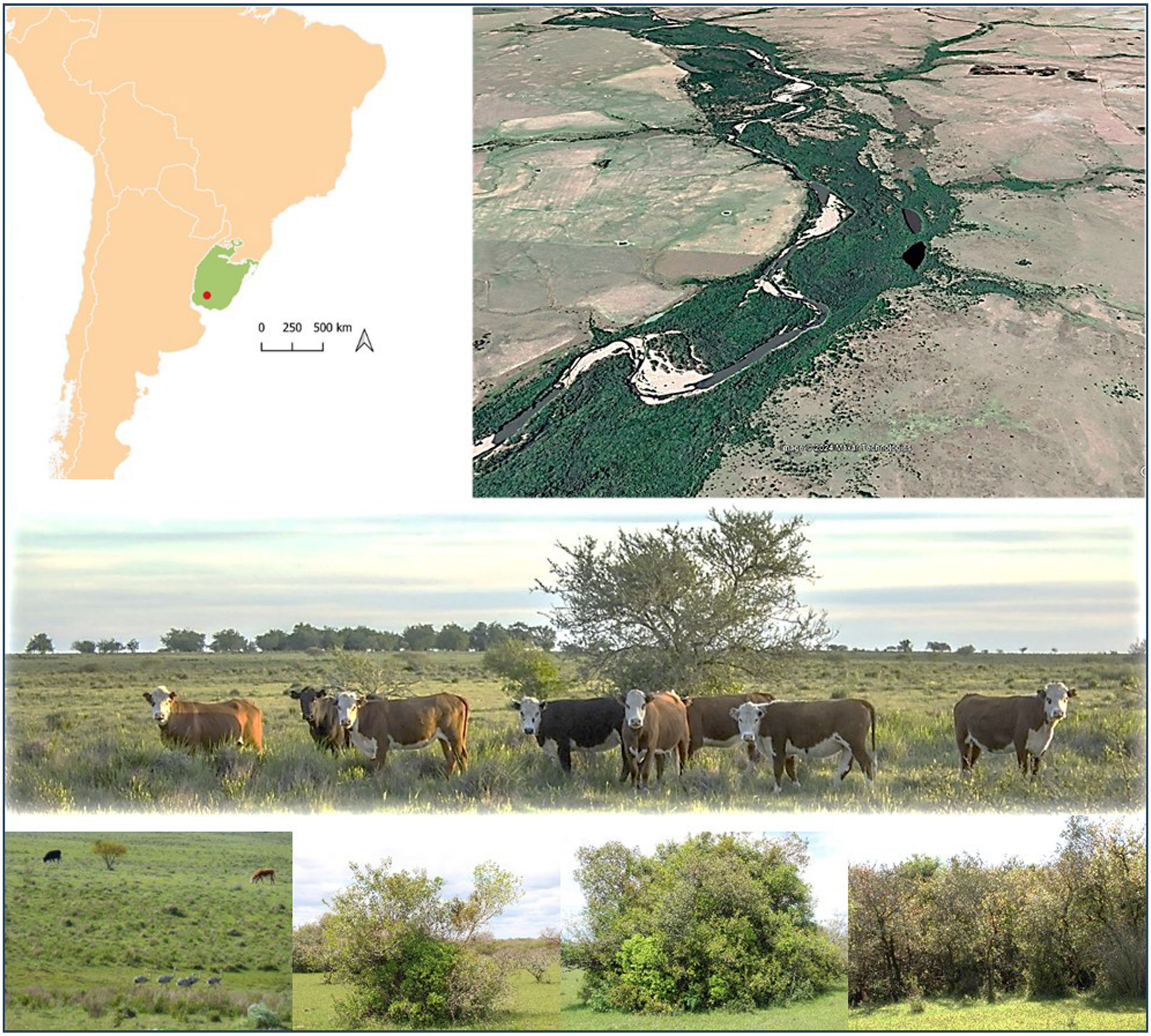


FIGURE 1 South America with the Campos ecoregion shaded (green) and the study site marked (red dot). Forests are restricted to the margins of the river Yi. The forest-grassland ecotone is shaped by the facilitative effects of nurse trees, especially the grassland tree *Vachellia caven*, and the herbivore effects of cattle. Forest tree species recruit under the canopy of nurse trees forming multi-specific tree patches. Tree patches of different sizes are found at the ecotone between grasslands and forests. But cattle ultimately limit the expansion of the forest (Photos: IR Hernandez-Salmerón, M Holmgren, and Google).

the riverine forest, we selected the sampling plots along the forest band: five meters from the river ($n=2$), middle of the forest ($n=2$) and five meters from the forest edge ($n=2$). Tree patches were sampled along a 300-m distance ecotone band from the forest edge into the grassland. Along this gradient, we sampled two replicates of each tree patch size category within 100-m blocks (except for big tree patches since they were present only up to 140 m. from the forest edge into the grassland). We used seed traps to assess seed inputs based on design by Muller-Landau et al. (2002). Seed traps were placed at approximately 75% radius of the patch tree canopy, always facing east towards the riverine forest. Seed traps were periodically monitored during the seed dispersal season (November 2015–January 2016).

We took one composite sample of the soil seedbank from each replicate. To have a representative sample of the soil seedbank, we sampled at 25% and 75% distance from the patch centre taking several subsamples per patch to ensure an equal sampling effort across tree patch size categories (Figure S2.1; Table S2.2). In grassland and forest systems, we took two subsamples per 25 m^2-replicate in adjacent areas to the tree patches ($n=6$ replicates). This sampling effort likely underestimates the soil seedbank of forests and grasslands but is used here as a reference to compare with soil seedbanks of adjacent tree patches. Soil subsamples were mixed well to extract one 900 cm^3 composite soil sample representative of each tree patch. Soil was dried at 85°C and sieved. We counted, identified, and measured all

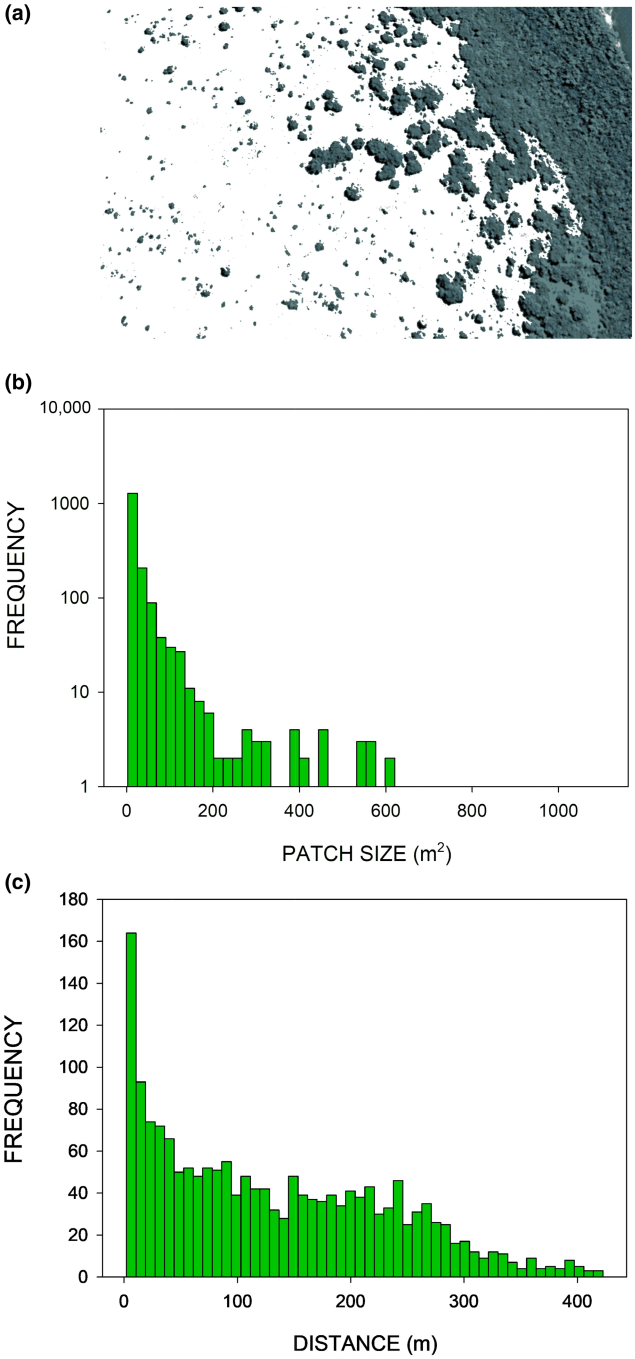


FIGURE 2 Tree patches cover the forest-grassland ecotone (a). Frequency of tree patches of different sizes (b) and at increasing distance from the forest edge (c).

seeds. Direct seed identification includes damaged or dormant seeds that may not germinate in seedling germination trails (Lindner, 2009).

2.3 | Environmental conditions at the forest–grassland ecotone

We assessed microclimate and soil nutrients in the six habitat types (grassland, four tree patch size categories and forest). Soil moisture and temperature were measured at 5 cm depth using a TRIME Soil Moisture Measurement Device (TRIME-PICO64, IMKO). We took four readings, halfway between the centre and the canopy edge, along a north–south transect to capture the potential spatial variation on abiotic conditions within the patch. We averaged readings to have one record per patch as there were no significant differences along the north–south transect within the tree patch canopy ($n = 12$ replicates for each habitat type). Sampling was conducted around midday hours (approximately 10:00–14:00) during consecutive days with sunny and dry weather (November 2015, November 2016). We sampled all six habitat types along this time interval to ensure comparable weather conditions.

We collected litter and soil samples from the six habitat types ($n = 6$ replicates per habitat). We placed a 25 × 25 cm frame to collect litter, dried it at 70°C and weighted. We took one composite soil sample from each replicate taking several soil subsamples per patch to ensure an equal sampling effort across patch size categories. Soil was extracted using a cylindrical auger with a diameter of 5 cm and height of 4 cm. Soil was dried at 70°C and analysed for total nitrogen and phosphorous content (g/g soil) following the protocols described by Valderrama (1981).

2.4 | Drivers of tree seedling recruitment

We conducted a field experiment at the forest–grassland ecotone to assess the roles of cattle, nurse trees and grass cover on tree seedling emergence and establishment. The experiment had a classical 2^3 nested design with each of the three experimental factors (herbivore, nurse tree and grass) at two levels (presence vs. absence) resulting in eight treatment combinations that were replicated 10 times, resulting in 80 1-m^2 experimental plots (Figure S3.1).

To exclude cattle, we built fences with electric wire. Inside every exclosure, we selected for solitary *V. caven* trees (approximately 4 m canopy diameter). We installed two plots under a tree canopy and two plots outside a tree canopy, at a distance of one canopy diameter towards the south. These 1-m^2 paired plots were separated at least 50 cm from each other. Grass cover was removed manually from one of the plots and maintained intact in the adjacent plot (both under and outside tree canopy). This experimental design was replicated outside each cattle exclosure in adjacent areas with similar characteristics.

In each 1-m^2 experimental plot, we buried seeds and planted seedlings of five native tree species with contrasting abundances in the forest–grassland ecotone (Muñoz et al., 2005): *Blepharocalyx salicifolius* (Arrayán), a species growing only in the forest; *Eugenia cisplatensis* (Guayabo colorado) and *Scutia buxifolia* (Coronilla), species found in both forest and grasslands as seedlings and adults; *Celtis tala* (Tala) and *V. caven* (Espinillo), species that occur more frequently in the grasslands and that are rare or absent in the forest. In each experimental plot, we sowed 10 seeds of each species and planted five seedlings of *S. buxifolia*, one seedling of

E. cisplatensis, one seedling of *B. salicifolius*, two seedlings of *V. caven* and five seedlings of *C. tala*. Differences in seedling number reflect differences in greenhouse emergence. We planted as many experimental seedlings as we were able to produce because seedling mortality is common. In summary, we planted 4000 seeds and 1120 seedlings across the 80 experimental plots. Seeds were collected from the region. Experimental seedlings were germinated from the same seed pool and grew for 2 months under same greenhouse conditions before transplanting. At planting time, seedlings were between 2.5 and 5.7 cm tall, reflecting intrinsic differences in growth rates across species. The field experiment was installed in October 2015 and monitored until September 2021. Seedling emergence, growth and survival were monitored periodically the first year because this is often the most sensitive ontogenetic phase of tree recruitment. We monitored survival and height of all surviving juveniles after 6 years.

2.5 | Statistical analysis

2.5.1 | Tree patches: Species composition, structure, functional traits

To assess tree patch size distribution from the forest edge into the grassland, we used a generalized linear model (GLM) with patch size as the dependent variable and distance to the forest edge as independent variable. We also used GLM to assess how tree communities (species richness, density, maximum height, DBH, community weighted functional traits) changed with patch size (m^2) and distance to the forest edge (m) as explanatory variables. We used binary logistic models to assess changes in dead trees (presence/absence) and spinescence (presence/absence) with same explanatory variables.

2.5.2 | Seed bank

We used one-way ANOVA to compare soil seed abundance and soil seed species richness across the six habitat types (grassland, four tree patch size categories and forest) and Fisher's LSD tests for post hoc comparisons between habitat types. We used GLM, assuming a Poisson distribution, to relate seed abundance and seed species richness to tree patch size and distance to the forest edge, including tree density and tree species richness as co-variables. We used a detrended correspondence analysis (DCA; Ter Braak & Smilauer, 2002) to further explore the species composition of the seedbank along the environmental gradients (distance to forest edge, distance to river, tree patch size, tree density and adult tree species richness). To compare diversity patterns across tree life stages, we computed Shannon–Wiener diversity index for seeds, seedlings and adults and compared them along the gradients of tree patch size and distance to river. We used Spearman's correlations to assess how species relative abundance across life stages of seeds, seedlings and adult trees were associated with each other.

2.5.3 | Environmental conditions

We used one-way ANOVA to assess microclimate (i.e. soil temperature and moisture) and soil variables (i.e. litter accumulation, nitrogen and phosphorus content) across the six habitat types. All response variables met the assumptions of normality and homogeneity of variance. Litter accumulation (g DW/0.0025 m^2) was transformed as ln $x+1$ to meet the assumptions.

2.5.4 | Field experiment: Seedling emergence, survival and growth

We used generalized linear mixed models (GLMMs) to assess seedling emergence and GLM to assess survival (with binomial distribution) and growth rate across experimental treatments after 1 and 6 years for each species separately. Fixed factors were 'herbivory', 'tree' (nested within 'herbivory'), 'grass' (nested within 'tree' which is itself nested within 'herbivory'). 'Replicates' was included as random factor. Grasses were only included during the first-year analysis when grass removal was periodically maintained.

3 | RESULTS

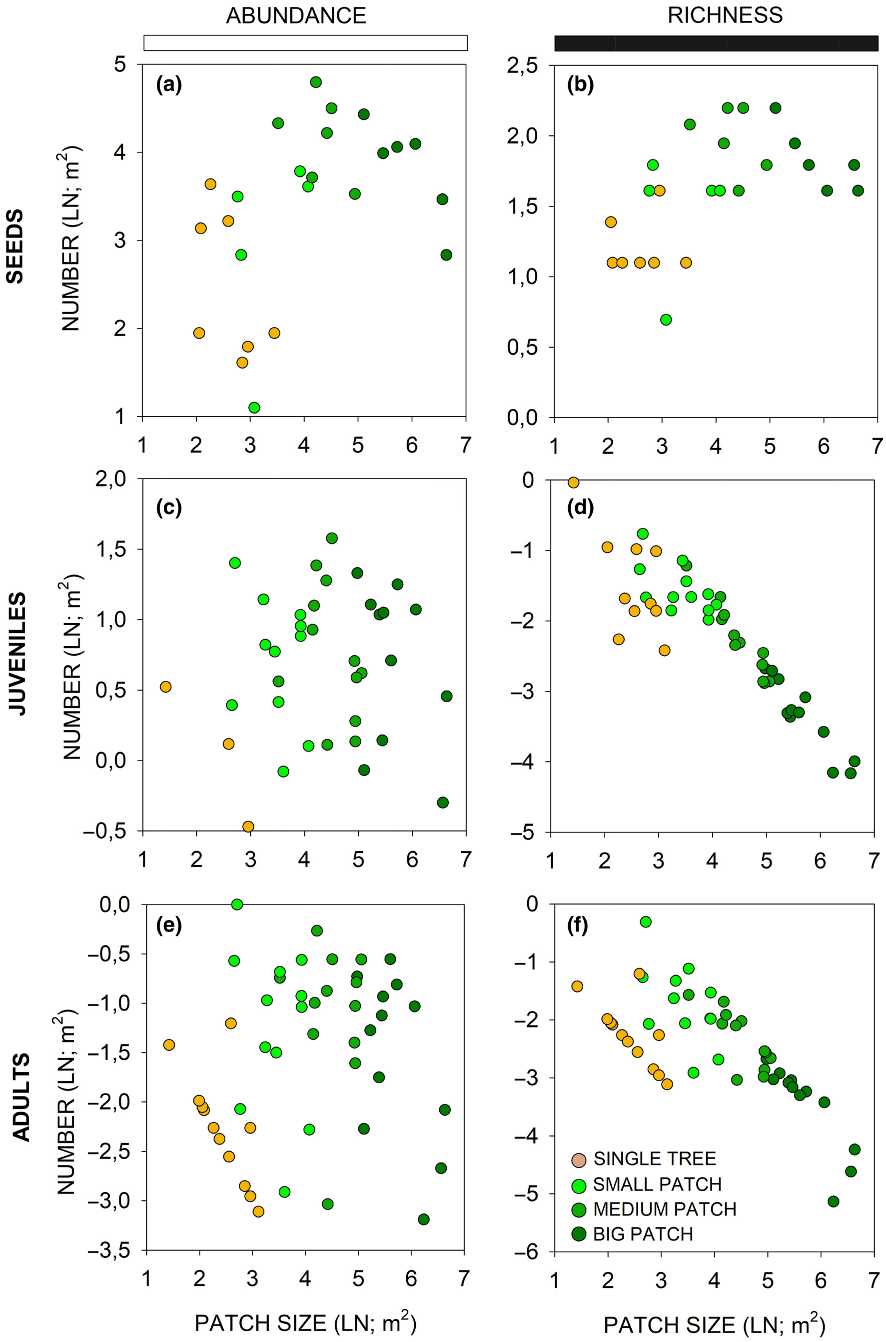
3.1 | Patterns of tree cover and species composition

Tree patch frequency decreased as patch size and distance from the forest edge increased (Figure 2b,c; GLM; $p=0.001$, respectively). Solitary trees and small patches were present along the whole forest–grassland ecotone, whereas large patches were mostly concentrated closer to the forest edge.

The structure of the tree community changed with patch size. Larger patches had taller and thicker trees (GLM; $p<0.001$, respectively) and tended to have a larger proportion of dead trees (GLM; $p=0.07$). The percentage of dead trees almost doubled with increasing patch size: 8% (solitary trees), 16% (small patches), 33% (medium patches) and 58% (big patches). The largest density of both juveniles and adults was reached within medium-sized patches (Figure 3c,e; GLM; $p<0.05$ and $p<0.008$). We recorded a total of 20 tree species within the tree patches (Table S1.2). Species richness of juvenile trees peaked under solitary trees while the highest species richness of adult trees was found in small tree patches (Figure 3d,f; GLM; $p=0.001$ respectively).

The seedbank was more abundant and diverse under tree patches than in grassland and forest, and peaked at medium-sized patches where adult tree density was highest (Figure 3a,b; ANOVA; $p=0.001$, respectively). Seed abundance was positively related with tree density (GLM; $p=0.008$). Seed species richness increased with tree density (GLM; $p<0.001$) and tree species richness (GLM; $p=0.026$) and decreased as the distance to the river increased (GLM; $p=0.05$). Seed rain and soil seedbank showed comparable patterns.

FIGURE 3 Species richness and abundance of seeds (a, b), juveniles (c, d), and adult trees (e, f) as patch size increases.



The multivariate DCA analysis indicates that occurrence of forest tree species in the seedbank is positively related to tree patch size and tree density, whereas seed occurrence of grassland tree species is negatively associated with these conditions. Seed occurrence of forest species increases closer to the forest edge and the river, whereas seeds of grassland tree species are found mostly further away. Seeds of generalist species occur along a broader range of environmental conditions (Figure S2.4).

Along the whole forest–grassland ecotone, over 90% of the solitary adult trees were *V. caven*. To explore changes in tree species composition, we classified tree species as: (1) generally growing in grassland, (2) generally growing in forest or (3) found both in grassland and forest (Muñoz et al., 2005; Table S1.2). The proportion of grassland tree species declined drastically as tree patches formed. Forest-grassland tree species were very rarely present as solitary trees but they were present in roughly equal numbers in small, medium and big patches (i.e. 35%, 30% and 35%, respectively). Forest tree species were never found growing as solitary trees. However, forest tree species represented 25% of the juveniles found under solitary trees and nearly 30% of the adult individuals found in small tree patches. As tree patches increased in size, the proportion of forest tree species increased whereas the proportion of grassland tree species decreased (Figure 4). Tree community average specific leaf area and leaf toughness tended to peak at intermediate sized tree patches, but trends are weak and statistically no significant. The proportion of trees with spines tended to linearly increase with patch size (GLM; $p=0.07$) (Figure S1.3).

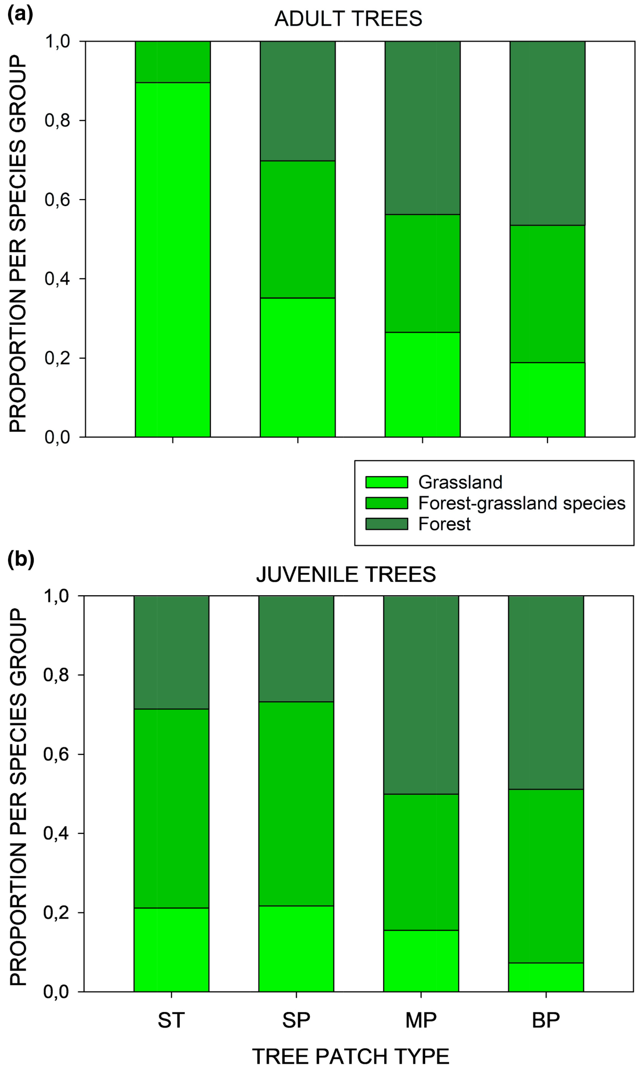


FIGURE 4 Proportion of adult trees (a) and juveniles (b) of grassland and forest tree species as patch size increases (BP, big patches; MP, medium patches; SP, small patches; ST, solitary trees).

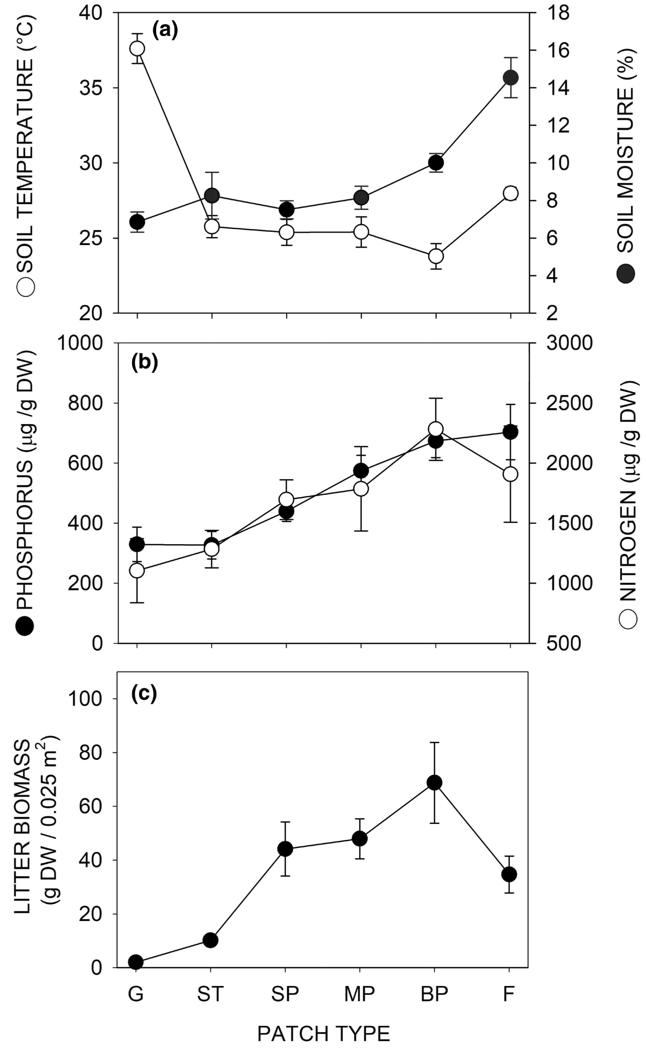


FIGURE 5 Microclimate (a), soil nutrients (b) and litter biomass (c) along a tree cover gradient including grasslands (G), solitary trees (ST), small patches (SP), medium patches (MP), big patches (BP) and forests (F).

Overall, the relative abundance of tree species found as seeds, seedlings and adults within the same patch were significantly correlated (Figure S2.5): seed and seedling life stages (Spearman $r = 0.127$, $p < 0.002$); seedling and adult life stages (Spearman $r = 0.453$, $p < 0.001$); seeds and adults (Spearman correlation $r = 0.364$, $p < 0.001$) (Figure S2.5). The species diversity in these three life stages rises rapidly with tree cover to peak on medium-sized patches (50–150 m^2) after which the diversity levels off or slightly decreases in the bigger patches.

3.2 | Environmental conditions at the forest-grassland ecotone

Grassland soils were warmer and drier than soils under tree patches and forest (Figure 5a; ANOVA; $p < 0.001$ for both variables). Soil nitrogen and phosphorous increased with tree cover (Figure 5b; ANOVA; $p = 0.06$ and $p < 0.001$, respectively). Litter accumulation and soil phosphorous also increased with tree cover (Figure 5c; ANOVA; $p < 0.001$).

3.3 | Experimental tree seedling emergence, survival and growth

3.3.1 | Germinated seedlings

Only 24% of all experimental seeds emerged successfully by the end of the first summer after 16 weeks since planting (February 2016). *V. caven* and *C. tala* were the most successful species ($68 \pm 1.7\%$ and $52 \pm 1.8\%$ of emergence, respectively) followed by *S. buxifolia* ($1 \pm 0.4\%$). Seedlings of *E. cisplatensis* and *B. salicifolius* did not emerge in any of the experimental treatments (Figure S3.2). Because seedling emergence rates differed across species (GLMM;

$p < 0.001$), we assessed the effects of cattle, nurse trees and grass cover separately. Tree canopy and cattle had no significant effects on seedling emergence. Removal of grass cover increased seedling emergence of *V. caven* seedlings and decreased emergence of *C. tala* (Figure S3.3).

Early survival rates of these germinated seedlings, during the first spring and summer, also differed across species: 71%, 48% and 27% for the seedlings of *V. caven*, *C. tala* and *S. buxifolia* (fa). Since these survival rates were significantly different (GLMM; $p < 0.001$), we also analysed the effects of cattle, nurse trees and grass cover separately for each species. Early survival of *V. caven* seedlings was reduced by tree shade, grass cover and cattle presence. In contrast, early survival of *C. tala* seedlings was favoured by tree shade and was not significantly affected by grass and cattle. Early survival of germinated *S. buxifolia* seedlings did not differ across experimental treatments.

3.3.2 | Survival and growth of planted seedlings

Survival of planted seedlings differed across species and experimental treatments (Figure 6; GLMM; $p < 0.05$; Figure S3.4). *V. caven* seedlings reached the highest survival rates of all species. Approximately 80% of the *V. caven* seedlings survived the first establishing year. Grass cover reduced seedling survival under nurse trees whereas cattle and trees had no significant effects. After 6 years, about 25% of the *V. caven* seedlings were still alive, especially in open grasslands. Seedling survival decreased under shade.

In contrast, seedling survival of all tree species, except *V. caven*, was higher under nurse trees. Grass cover increased their survival especially when growing outside nurse trees. After 6 years, seedling survival rates of all species, except *V. caven*, were highest under trees and in the absence of cattle. In open grasslands, most seedlings had died. Seedling establishment of *B. salicifolius* and *E. cisplatensis* were most difficult and only few seedlings remained alive under the shade of nurse trees after 6 years.

Seedling growth rates also differed across species and experimental treatments (GLM; $p < 0.01$). *V. caven* seedlings grew fastest since the first year, especially without cattle, outside nurse trees and with no grasses (Figure S3.6). After 6 years, *V. caven* had the tallest juveniles of all tree species, especially in open grassland without cattle (Figure S3.7). In general, seedling growth of the remaining species was less affected by the experimental treatments the first year. Growth of *C. tala* was reduced by grass cover and cattle but unaffected by shade. Growth of *S buxifolia* was higher under trees in the absence of cattle, and also when grasses were removed outside nurse trees. Survival after 6 years was too low to allow assessing treatment effects.

4 | DISCUSSION

Our results demonstrate that tree cover expansion from the riverine forests into open grasslands of the Campos is limited by cattle browsing but can be facilitated by nurse trees, especially *V. caven*. Although cattle and grass cover reduce the emergence and early survival of *V. caven* seedlings, this tree species recruits successfully representing up to 90% of all solitary trees in the grassland. Similar patterns were found in subtropical wet Savannas of northeast Argentina (Macias et al., 2014).

The recruitment success of *V. caven* in grasslands may be explained by positive effects of cattle on seed dispersal, seedling germination and early seedling establishment. Cattle are important seed dispersers of *V. caven* (Velasco et al., 2023). In addition, cattle dung could act as a safe microsite, with moist and nutrient rich conditions and low grass competition, that facilitates tree seedling establishment, as found in other Savanna systems (Brown & Archer, 1988; Miceli-Mèndez et al., 2008). This early advantage in growing conditions may open a window of opportunity for this fast growing species (Aronson et al., 1992), allowing shoots to escape from cattle browsing and roots to reach deeper soil layers with low grass root competition (Scheffer et al., 2008).

Our results show that once *V. caven* establishes, it facilitates the formation of tree patches. Juveniles of most tree species establish under the canopy of solitary *V. caven* trees. This facilitative role of *V. caven* trees may result from both seed accumulation and increased seedling survival under their canopies. Frugivorous birds feed on many forest tree species and often perch on trees sparsely growing in the open grassland, thus promoting tree colonization (Duarte et al., 2006; Pozo Inofuentes & Säumel, 2022). Indeed, we found a larger abundance and diversity of tree seeds in the soil seedbank under isolated trees and tree patches than in the open grassland. Seed rain likely depends on the traits of the perching trees. For example, tall *V. caven* trees, with larger canopies, attract a larger input of forest tree seeds than smaller *V. caven* individuals (Miranda et al., 2019). In our experiments, seedlings of all tree species, except *V. caven* itself, survived longer and grew faster under the canopy of adult *V. caven* trees.

A nursing role of *V. caven* on the recruitment of forest tree species has also been found in drier ecosystems, such as the dry forests of Uruguay (Pozo Inofuentes & Säumel, 2022) and the evergreen sclerophyllous forests of central Chile (Fuentes et al., 1986; Gómez-Fernández et al., 2023; Root-Bernstein et al., 2017). Continental comparisons on the facilitative role of *V. caven* on perennial plants show that facilitative effects change along precipitation gradients, peaking at intermediate rainfall levels in the western Andes whereas increasing linearly with precipitation in the eastern side of the Andean range (Velasco et al., 2024), a pattern consistent with theoretical predictions on the relative importance of facilitation along abiotic stress gradients (Holmgren & Scheffer, 2010). Locally, nursing effects are often found to be related to microclimatic effects. Possibly, the higher C3/C4 grass ratio under *V. caven* trees played a role too, as C3 grasses favoured by the microclimate conditions found under the canopy of *V. caven* trees (Bernardi, de Jonge, & Holmgren, 2016; Fernandez et al., 2014), are less competitive than C4 grasses.

Surprisingly, grasses had negative effects on emergence, growth and survival of *V. caven*, the most successful species in the grassland.

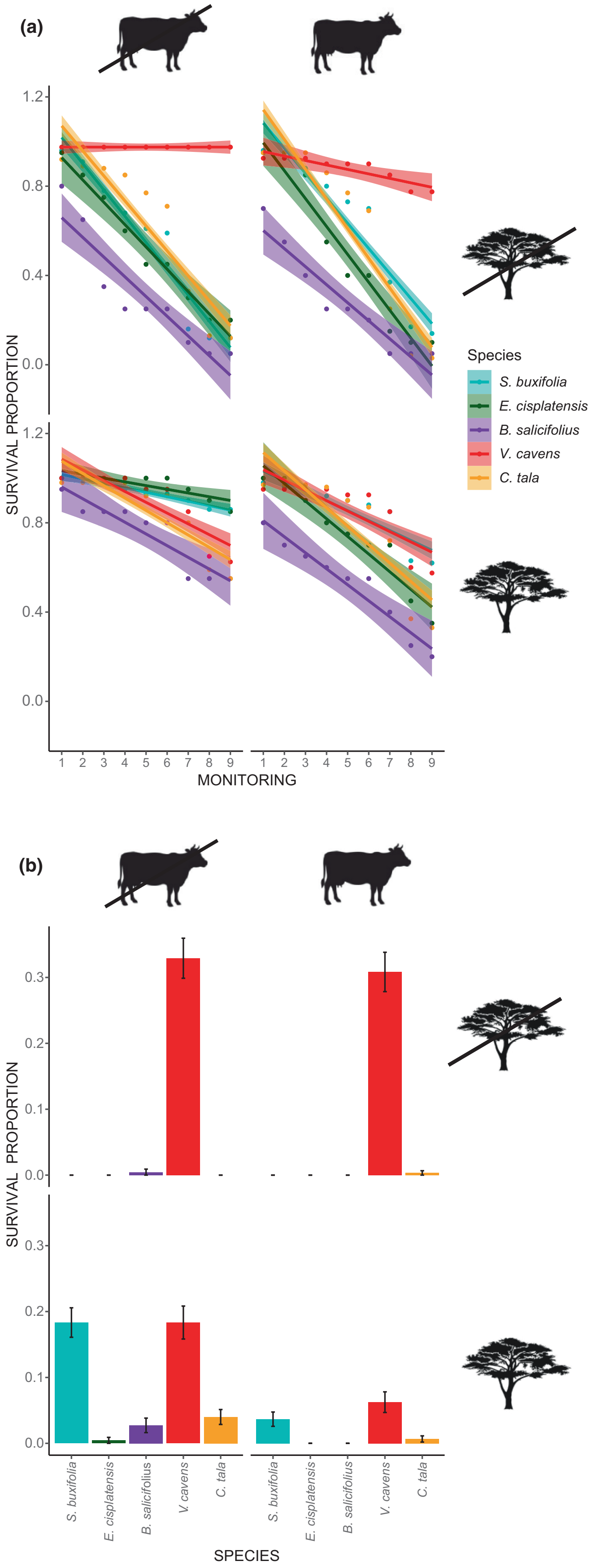


FIGURE 6 Effects of cattle and nurse trees on survival of experimental seedlings during the first year (a) and sixth year (b).

Meanwhile, grasses promoted emergence and establishment of *C. tala*, and seedling survival of *S.buxifolia*, *E. cisplatensis and B. salicifolius* possibly due to abiotic stress amelioration. We observed healthy tree seedlings under the dense grass canopy of the experimental treatments. Indeed, although most literature emphasizes the competitive effects of grasses on tree seedlings, grasses may also reduce abiotic stress and herbivore damage (Anthelme & Michalet, 2009; Good et al., 2014).

As tree patches grow, they become increasingly dominated by species generally associated with forests, plausibly favoured by a shady environment with cooler summer temperatures, moister soils, and a thicker layer of litter enhancing water retention and nutrient availability. Tree patches may ameliorate extreme temperatures in winter too reducing the negative effects of frost on seedlings and saplings (Araujo Frangipani et al., 2021). The higher proportion of forest tree species is not reflected in changes in the community mean specific leaf area or leaf toughness perhaps due to cattle browsing effects. Such a role for browsing is consistent with the increased proportion of spiny trees we find in the largest patches.

Our finding that *V. caven* colonizes grazed grassland and subsequently promotes the development of forest patches, raises the question why not all those grasslands are turning into forest. Clearly, forest expansion stalls. Patches are formed but do no develop into continuous forest. One possible explanation is that patches become more vulnerable to destruction by cattle as they grow. Small tree patches form a dense barrier that impedes cattle browsing and trampling. As the patches become larger, they also become less dense, making it easier for cattle to enter and use the patches for shelter at night and during hot and cold days (i.e. personal observations). Eventually, this may lead to disintegration of the larger tree patches. Closer to the river, tree patches are larger. Here, microclimate and seasonal flooding provide more benign environmental conditions for riverine forest tree species to grow (Mohan et al., 2022). Higher tree growth rates in those environments may balance negative cattle effects. Thus a decline of growth rates with increasing distance to the river may limit forest expansion in those grazed landscapes.

The effects of browsers as controllers of tree cover, and nurse trees as facilitators of tree expansion, have also been described for southern Brazilian grasslands (Müller et al., 2023) and eastern Uruguayan hillsides (Brazeiro et al., 2018). Thus, the interplay of those mechanisms over the gradient of habitat suitability may well shape the striking pattern of river-associated forest distribution in the South American Campos. It remains an open question whether cattle exclusion would lead to natural forestation of those landscapes. The positive effect of cattle on nurse species *V. caven* would be lost in such a setting. On the other hand, spontaneous settlement of native tree species in cattle free zones such as road verges suggests the potential for a forested landscape in places where cattle is restricted.

Even under present grazing levels, increased rainfall as climate change progresses in this region (Torres et al., 2022) may further facilitate tree cover expansion (Bernardi, Holmgren, et al., 2016; Bernardi, Staal, et al., 2019; Holmgren et al., 2013; Lucas et al., 2017; Staal et al., 2018). Trees in productive pastures have traditionally been perceived as a threat to grassland productivity as tree shade can reduce grass growth. However, growing evidence shows that sparse tree cover can contribute to climate change adaptation of productive systems by increasing grass availability (Hernández-Salmerón et al., 2023; Hernández-Salmerón & Holmgren, 2022) and ameliorating livestock heat stress during hot and dry conditions (Lins et al., 2021; Veissier et al., 2018). Clearly, tree cover in those landscapes has a range of costs and benefits when it comes to ecosystem functions ranging from food production to biodiversity and carbon storage. Quantifying the associated trade-offs in ecosystem services may help resolving the often passionate debate on a big question 'To tree or not to tree' (Holmgren & Scheffer, 2017).

AUTHOR CONTRIBUTIONS

Milena Holmgren conceived the ideas. Alice Blok, Jasper Buijs, Ivan Raniero Hernández-Salmerón, Milena Holmgren, Rocío Martínez-Cillero, Nestor Mazzeo and Bart Verdijck designed methodology, collected data and analysed data. Edwin Bargeman and Rafael Bernardi collected data. Milena Holmgren led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication. Our study resulted from the long-term collaboration between Wageningen University (The Netherlands) and the South American Institute for Resilience and Sustainability Studies (Uruguay).

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CONFLICT OF INTEREST STATEMENT

Authors declare no conflicts of interests.

DATA AVAILABILITY STATEMENT

Data available from the DANS Digital Repository: https://doi.org/10.17026/LS/UMSOXN (Holmgren et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1: Tree patches: Location, species composition and functional traits.

Appendix S2: Seed rain and seedbank assessments.

Appendix S3: Field experiment: Design and survival analyses.

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