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ORIGINAL PAPER

Antagonistic effects of large- and small-scale disturbances on exotic tree invasion in a native tussock grassland relict

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Abstract It is generally accepted that disturbances increase community invasibility. Yet the role of disturbance in plant invasions may be less predictable than often assumed, due to the influence of environmental stochasticity and interactions between disturbance regimes. We evaluated the single and interactive effects of prescribed burning (large-scale, infrequent event) and animal diggings (small-scale, frequent events) on the invasion success of *Gleditsia triacanthos* L. in a tussock grassland relict of the Inland Pampa, Argentina. Tree seedling emergence and survival were monitored over 4 years, after adjusting for propagule pressure through copious seed addition to all disturbance treatments. Burning altered community structure by suppressing tussock grasses and promoting exotic forbs, whereas simulated, armadillo-like diggings had little impact on herbaceous composition. Overall, seedling emergence rather than survival represented the main demographic bottleneck for tree invasion. Tree establishment success varied among seedling cohorts emerged in different climatic years. In a dry year, emergence was only slightly affected by disturbances. In contrast, for two consecutive wet years, initial burning and armadillo-like diggings exerted strong, antagonistic effects on tree recruitment. Whereas fire alone increased recruitment, the simulated burrowing regime prevented seedling emergence in both burned and unburned plots. The latter effect might be explained by reduced soil moisture, and increased seed burial and predation in excavated patches. Thus, the impact of a single, large-scale perturbation promoting woody plant invasion was overridden by a regime of small-scale, frequent disturbances. Our results show that grassland invasibility was contingent on inter-annual climatic variation as well as unexpected interactions between natural and anthropogenic disturbance agents.

Keywords Animal burrows · Fire · *Gleditsia triacanthos* · Seedling recruitment · Seed predation · Tree–grass interactions

Introduction

A well-known generalisation states that disturbances promote species invasions into otherwise ‘resistant’ communities (Elton 1958; D’Antonio et al. 1999; Lockwood et al. 2007). Disturbances are discrete

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physical or biotic forces that alter community structure by damaging resident organisms or by altering their substrate or resource base (White and Pickett 1985; D'Antonio et al. 1999; Svensson et al. 2009). Disturbances may increase community invasibility by making resources available to non-resident species through competitive relaxation or consumer release (Hobbs and Huenneke 1992; Davis et al. 2000; Shea and Cheeson 2002). However, disturbance effects on invasibility are often compounded by differences in exotic species pools, propagule pressure, and disturbance regimes (Williamson 1996; Lonsdale 1999; D'Antonio et al. 1999; Lockwood et al. 2007). Moreover, even relatively stable, undisturbed systems may be invaded under high propagule pressures (Williamson 1996; von Holle and Simberloff 2005). It is therefore critical that effects of various disturbance regimes be evaluated singly and in combination, while controlling for invader identity and propagule availability.

The frequency and spatial scale of disturbances (i.e. their 'regime'; White and Pickett 1985; Svensson et al. 2009) introduces a major source of variation in community invasibility (Hobbs and Huenneke 1992; D'Antonio et al. 1999). Plant communities affected by recurrent, small-scale disturbances such as soil removal by burrowing animals may be relatively resistant to invasion if canopy gaps are rapidly closed by remnant plants (Davis et al. 2000). Yet native plant communities are often dominated by slow-growing perennial species, which exhibit slow recovery rates after severe disturbances (Alpert et al. 2000; Grime 2001). On the other hand, a community affected by infrequent, large-scale events such as burning may be prone to colonisation by novel species and to shifting into alternative states (Petraitis and Latham 1999). By considering that post-disturbance resource pulses are transient, a key point is whether resident plants will recapture resources before invaders are able to profit from them (Davis et al. 2000; Shea and Cheeson 2002). Unfortunately, few studies measure recovery rates from differently-scaled disturbances in the same ecosystem (Petraitis and Latham 1999).

The impact of disturbance on community invasibility has been claimed to be context-dependent (Lockwood et al. 2007). Disturbances may create idiosyncratic patterns of invasion due to interactions between biotic and abiotic forces (Crawley 1989; Chaneton and Facelli 1991; D'Antonio 1993; Chaneton et al. 2002). The combination of disturbances acting at different spatial scales may synergistically promote invasion, for instance, when small-scale events create resource hotspots in a community matrix altered by a large-scale perturbation (Hobbs and Huenneke 1992; McIntyre and Lavorel 1994). Conversely, effects of different agents may be antagonistic, so that exotic species success is decreased under mixed disturbance regimes (Chaneton et al. 2002). In addition, the extent of invasion can be influenced by stochastic environmental variation (Crawley 1989). High rainfall years may facilitate invasion by creating 'windows' of opportunity for recruitment of novel species (O'Connor 1995; Davis et al. 2000). Interactions between disturbances and weather conditions may also be complex (Alpert et al. 2000). While disturbances release resources pre-empted by resident plants, extreme conditions can either obliterate such resource pulses (drought year) or further increase resource availability to colonisers (humid year) (O'Connor 1995; Davis et al. 1998; Davis and Pelsor 2001).

The local community context includes the role of consumers as potential barriers to invasion (Maron and Vilà 2001; Mitchell et al. 2006). There is growing evidence that exotic plants can be challenged by resident generalist consumers, including granivores and seedling predators that prevent recruitment during early stages of an invasion (Levine et al. 2004; MacDougall and Wilson 2007; Nuñez et al. 2008). Moreover, consumers may influence invasion indirectly, through the presence of third parties (White et al. 2006). For example, herbivores or pathogens thriving in community gaps created by burrowing mammals may limit gap exploitation by invaders (D'Antonio 1993; Eviner and Chapin 2003). Such indirect effects represent an underestimated mechanism of resistance to invasion (Mitchell et al. 2006; White et al. 2006). Overall, large-scale anthropogenic disturbances are expected to alter consumer impacts on exotic species through habitat destruction (Elton 1958).

In this study we tested how different disturbance regimes, singly and interactively, influenced seedling emergence and survival of the invasive tree *Gleditsia triacanthos* L. (Fabaceae, Caesalpinacea) in a native tussock grassland relict, in the Inland Pampa of Argentina. We compared the effects of two common disturbances, namely, prescribed burning and soil removal by burrowing mammals, which have characteristically different regimes regarding the frequency and spatial extent of events (Hobbs and Huenneke 1992; D'Antonio et al. 1999). To adjust for seed availability as a factor limiting invasion (Mazía et al. 2001), we homogenised propagule pressure across disturbance treatments by adding large quantities of *G. triacanthos* seeds in different climatic years. Lastly, we examined the impact of resident granivores on tree seed survival as affected by disturbance context. Specifically, we asked the following questions:

(1) How does disturbance regime affect tree seedling recruitment into native grassland?
(2) How does invasibility change when biotic and abiotic disturbance agents operate at different scales on the same plant community?
(3) To what extent do disturbance effects on tree invasion differ for seedling cohorts emerging in climatically contrasting years?
(4) What is the influence of disturbance regime on tree seed removal by granivores?

Methods

Study system

The study was conducted near Ordoqui, Carlos Casares county, 400 km west of Buenos Aires, Argentina (36°S, 61°05′W). Mean annual rainfall is 1,016 mm (SD = 266 mm, 1976–2005); mean temperatures range between 7.2°C in July and 23.8°C in January. The dominant soils are well-drained Hapludolls. Mesic tussock grasslands were the original vegetation type, comprising a species-rich array of perennial grasses (Parodi 1947; Soriano 1992). The Inland Pampas have been extensively transformed to row-crop agriculture and livestock grazing. Native tussock-grass communities are nowadays confined to small relictual fragments and narrow corridors along abandoned railway lines and secondary road verges (Soriano 1992; Ghersa and León 1999). Remnants are seasonally grazed by cattle and are also sporadically burned to improve forage production (Laterra et al. 2003).

Woodlands did not originally occur in the mesic pampas before European settlement (Soriano 1992). Many introduced tree species have escaped from cultivation and became invasive, and may now be found invading old fields, gardens, fence lines, and roadside corridors (Facelli and León 1986; Mazía et al. 2001; Ghersa et al. 2002). *Gleditsia triacanthos* (honey locust) is a deciduous tree native to the eastern USA (Schnabel and Wendel 1998), which was presumably introduced to the region in the mid 1800s as an ornamental and shade plant, and was also used to build living fences (Hudson 1918). It is a dioecious, insect-pollinated tree, producing large indehiscent legumes with up to 20 seeds (seed mass *c.*200 mg), which are readily dispersed by cattle (Marco and Páez 2000) and probably by native fauna as well (e.g. foxes, armadillos). Adult trees may form dense, thorny thickets with up to *c.*1,600 trees/ha, which strongly reduce herbaceous productivity and impede livestock treading (M. I. Miranda and E. J. Chaneton, unpublished manuscript). *Gleditsia triacanthos* has several life-history traits providing high invasive potential, including large seed crops, high germinability, rapid growth, and a short juvenile stage (Burton and Bazzaz 1991; Marco and Páez 2000). It has been reported as invasive around the world (Global Compendium of Weeds: http://www.hear.org/gcw/index.html), being nowadays the most aggressive and widespread woody invader in the mesic pampas (Mazía et al. 2001; Ghersa et al. 2002).

The study site was a 400 m-long by 60 m-wide strip of a tussock grassland corridor extending for 10 km parallel to an abandoned railway line. The site was surrounded by crop fields and rangelands and was not heavily grazed or burned for at least 20 years. The plant community was dominated by *Paspalum quadrifarium* Lam., a tall C_4 perennial bunchgrass accounting for 90% of the total standing biomass. Other species comprised the exotics *Festuca arundinacea, Carduus acanthoides* and *Dypsacus sativa*, and a diverse mix of native grasses including *Briza subaristata*, *Stipa neesiana*, *Bothriochloa laguroides* and *Melica brasiliana* (nomenclature follows Cabrera and Zardini 1978). The grassland had a coarse-grained structure, with large grass tussocks embedded in a thick litter layer (*c.*800 g/m^2; Machera 2006). Two *G. triacanthos* adult trees were already established along the grassland corridor.

Experimental treatments

The experimental area was fenced in summer 1999 to exclude burrowing mammals. In March 1999, we set up 20 plots (8-m × 11-m each) and randomly assigned them to four treatments in a 2 × 2 factorial design ($n = 5$). Plots were separated by 10-m wide buffer zones. In each main plot, a 3-m × 4-m ‘subplot’ was delimited for use in this study (Fig. 1). We created two disturbance regimes: (1) *prescribed burning*, a large-scale, infrequent anthropogenic disturbance, normally applied in winter–spring once every 3–5 years (Laterra et al. 2003); and (2) *armadillo diggings*, a small-scale, recurrent natural disturbance produced by native fossorial mammals (*Chaetophractus villosus*) throughout the year. The burning treatment was applied in May 1999. Fire removed most above-ground biomass, leaving only some large tussock bases. Both the timing and severity of fire resembled those used by land managers in the Pampas (Laterra et al. 2003).

Armadillo-like disturbances were applied bi-monthly at the subplot scale (Fig. 1), over two growing seasons (May 1999 to December 2000). Armadillo diggings (burrow + mound) were simulated by removing all plants and soil to 15-cm depth, and turning the soil to create a small mound. Each digging was 0.12 m^2 (30 × 40 cm) in size, similar to fresh armadillo burrows (0.14 ± 0.05 m^2, mean ± SD, $n = 20$; Machera 2006). We created three armadillo diggings per subplot per date, for a total of nine disturbance dates. This regime was derived from a year-long survey of natural burrows in the study area (M. L. Bolkovic, unpublished data). Diggings were located at random with the constraint that no patch could be disturbed twice. The total area affected by our digging regime represented 30% of the subplot. In the unburned grassland, diggings altered the litter layer between grass tussocks, but did not kill established plants. In the burned plots, however, diggings often killed several weedy forbs that emerged after the fire (Machera 2006).

Seed addition and seedling monitoring

We evenly added 40 seeds/m^2 to each subplot (Fig. 1) after each armadillo disturbance event, for a total of nine sowing dates. Tree legumes were collected in autumn from nearby patches; seeds were removed by hand, stored dry, and gently scarified before sowing. We added a total of 3,240 seeds per subplot, and 64 800 seeds for the whole experiment. This sowing protocol effectively homogenised the propagule pressure among treatments (Simberloff 2009), allowing us to focus on limitations to tree recruitment based on microsite availability (Davis and Pelsor 2001; Mazía et al. 2001).

Sampling took place between October 1999 and March 2003. Monitoring was performed on a monthly basis during spring–summer of two study seasons (1999–2000 and 2000–2001), but was limited to summer for the remaining of the study. On each sampling date we mapped, counted and marked all tree seedlings emerging within sown areas using colour-coded cocktail sticks. We also recorded survival of plants marked in prior surveys. Within each main plot, an area equivalent to the sown subplot was thoroughly searched to account for background germination, but no tree seedling was found in unsown areas. We did not score mortality causes, although observations indicated plants could be killed by abiotic stress, herbivory or physical damage by litter deposition. Seedling emergence began in mid-spring (October) but peak emergence varied between years. We followed three plant cohorts that emerged during October 1999–March 2000 (cohort 2000), October 2000–March 2001 (cohort 2001) and November 2001–March 2002 (cohort 2002). Seed addition was discontinued in December 2000, although seedlings kept emerging from remnant seeds over the next 2 years (cohorts 2002 and 2003). The latest cohort (2003) was disregarded because seedling numbers were too low.

Growing-season (October–March) precipitation was 11, 75 and 39% higher than average (690 mm, 1976–2005) for 1999–2000, 2000–2001 and 2001–2002, respectively. However, the first spring (September–November 1999) was drier than usual (298 mm rainfall, i.e. 22% below average), although it was followed by a wet summer (January–March 2000, 358 mm, 62% above average). The second and third study seasons were uniformly wet (both spring and summer rainfall >20% above average). Total rainfall for the last study season (2002–2003) was also higher (45%) than average.

Disturbance effects on grassland structure

In December 2000, we sampled the grassland community composition using two 1-m^2 quadrats placed in each subplot (Fig. 1). The percent cover of all vascular plant species was visually estimated to the nearest 5%. To assess vegetation recovery after fire, in March 2000 and 2002, we measured aboveground biomass in burned and unburned plots without diggings ($n = 5$). Three quadrats (50 × 20 cm) were randomly located inside the main plot area (see Fig. 1) and all aerial biomass including the litter was collected, dried at 70°C and weighed; quadrats within plots were averaged for analysis. Percent biomass recovery was calculated as the ratio between the average biomass in burned and control plots.

We also assessed recovery rates from simulated armadillo disturbances at different times of year, starting in May, August, October, and December 2000. On each initial date, three armadillo-like diggings were paired with adjacent undisturbed controls in the main area of burned and unburned plots ($n = 5$; Fig. 1). Paired control and disturbed microsites (0.12 m^2) were sampled for aboveground biomass after 3–7 months (intervals varied with season) and data were used to calculate mean monthly recovery rates (3 microsites × 4 dates) per plot, expressed as percent biomass of disturbed relative to control microsites.

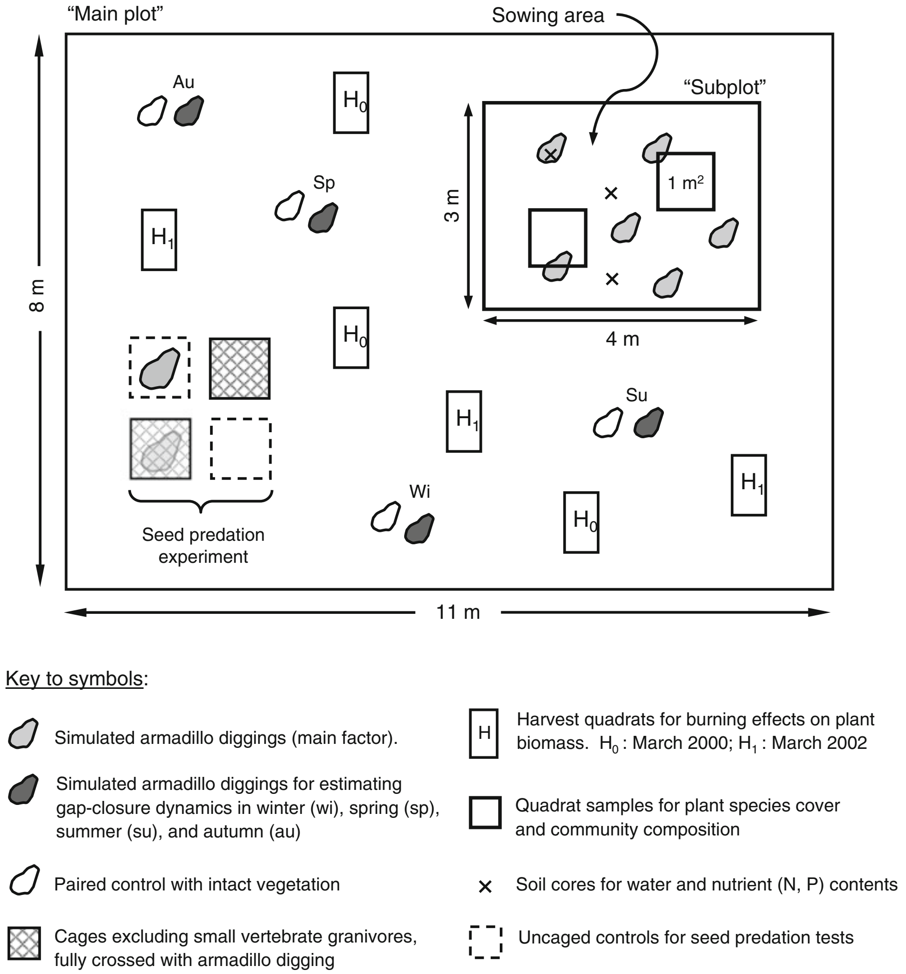

Fig. 1 Layout of experimental manipulations and measurements within a study plot. Burning treatments (burned/unburned) were applied at the main-plot scale and simulated armadillo diggings (with/without) were created at the subplot scale, in a fully randomised factorial design ($n = 5$). Establishment of *Gleditsia triacanthos* was monitored within each subplot sown at 40 seeds/m^2. Figure not drawn at scale. See text for details

Light, water and nutrient availability

In early December 2000, we measured percent light penetration through the canopy. Photosynthetically active radiation (PAR) was recorded at ground level and above the canopy for seven random points outside the main plots, using an integrating quantum sensor (LICOR 188-B, Nebraska, US). We were unable to monitor changes in light conditions during post-fire vegetation recovery. Light levels within armadillo diggings were measured by placing the PAR sensor at the centre of 12 artificial mounds and beneath the adjacent plant canopy. Light readings were taken at midday (12:00–14:00) during clear days. In December 2000, we measured effects of burning and armadillo gaps on soil water, nitrogen (N) and phosphorus (P) contents. In each subplot, three 6-cm diameter × 15-cm deep soil cores were extracted and pooled for analysis (Fig. 1). In the armadillo-disturbed plots, samples were taken from freshly created mounds, while in other plots soil samples were extracted at random from the vegetation matrix. Soil subsamples were extracted with a 2 M KCl solution; extracts were assayed for ammonium and nitrate concentrations in an Alpken autoanalyser (Wilsonville, Oregon). Another subsample was used to determine soil extractable P (Bray-Kurtz). A third subsample was used to measure soil gravimetric water content (%) after drying at 105°C.

Seed removal by granivores

Tree seed loss to resident granivores was assessed in a side experiment using rodent exclosures. In each main plot with armadillo disturbances (control and burned, $n = 5$), we established a four-treatment block with rodent exclusion (with or without cage) and armadillo digging (with or without) as main factors (Fig. 1). Exclusion cages (30-cm diameter × 40-cm height) were made of 2-cm mesh wire screen attached to the ground with iron pegs. Armadillo disturbances were simulated as described before. In each microsite we placed a 9-cm diameter dish containing sieved soil and 20 seeds of *G. triacanthos*. The experiment was repeated in June, August and September 2000. We counted the number of intact seeds and of those damaged by invertebrate consumers. Seed removal by rodents was computed as the difference in seed loss between caged and uncaged microsites for each armadillo treatment. Missing or damaged seeds within cages allowed us to account for invertebrate predation (insects and slugs). Live trapping showed that *Akodon azarae* was the most abundant granivorous rodent in the study site (>85% of all captures; M. Busch et al. unpublished data).

Statistical analysis

Changes in grassland community structure were evaluated by two-way ANOVA, with burning and armadillo disturbances as main effects. The cover of different plant groups was the response variable; cover data were arcsine-square-root transformed for analysis. Tree seedling emergence and establishment per plot were analysed using generalised linear models (Crawley 1993), with burning (2 levels), armadillo digging (2 levels) and plant cohort (3 levels) as main effects ($n = 5$). For each cohort, the peak number of seedlings found in summer was used as a proxy of total emergence, while seedling establishment was computed as the number of plants surviving at the end of the study (March 2003). Emergence and establishment were count data, which are usually modelled assuming a Poisson error structure (Crawley 1993). In our case, however, models with Poisson errors were strongly over-dispersed and yielded large residual deviances. Instead, model fitting was best achieved assuming a Gaussian error distribution after log $(x + 1)$ transformation of the original data. Disturbance by cohort interactions had a substantial impact on model deviances ($P < 0.10$); then, we tested for disturbance effects within cohorts, with P values adjusted for multiple tests (Bonferroni's $k = 3$, i.e. $P < 0.0167$). Disturbance effects on seedling survival were evaluated for each separate cohort using log-linear analysis, with plants pooled over replicate plots within treatments. Survival reflected the number of plants alive in March 2003, relative to the peak emergence of each cohort.

Soil water content and nutrient levels were evaluated by two-way ANOVA including burning and armadillo diggings as main effects, and with angular or logarithmic data transformations, respectively. Total seed removal was analysed by repeated-measures ANOVA using a split-plot factorial design. Burning was entered as the main-plot factor, while armadillo digging and rodent exclosure represented two, fully-crossed, subplot factors. For analyses of seed removal by rodents, the cage effect was excluded because the difference between caged and uncaged dishes was used as the response variable. Likewise, only caged dishes were considered in testing for seed loss to invertebrates. The date of experiment (3 levels) was the repeated measure. Percent removal data (%) were arcsine-square-root transformed before analysis. All statistical analyses were implemented using STATISTICA for Windows (StatSoft Inc. 1999).

Results

Disturbance effects on grassland community structure

Based on the above-ground biomass of control plots, initial burning removed an estimated 1894 ± 43 g dry mass/m^2. Recovery from fire was a slow process in this tussock-grass community. At the end of the first growing season (March 2000), above-ground biomass in burned plots was about 26% that in control plots, and after 3 years (March 2002), it had recovered to only 42%. In addition, burning reduced litter mass to less than 20% of that in control plots. Mean monthly rates of biomass recovery after simulated armadillo diggings were similar for unburned (14.7 ± 2.7%) and burned (13.3 ± 2.9%) grassland. Total standing biomass in armadillo-like gaps fully recovered after 6 months from disturbance.

Effects on grassland composition differed between disturbance treatments (Fig. 2). Burning strongly suppressed the dominant grass *P. quadrifarium* (Fig. 2a), whereas it increased the cover of forb species as well as of subordinate native and exotic grasses (Fig. 2b, c). Armadillo-like diggings increased forb cover but reduced that of subordinate grasses, especially in burned plots (Fig. 2b, c).

Disturbance effects on tree recruitment

Seed addition generated three plant cohorts that emerged in summer 2000, 2001 and 2002 (Fig. 3). The timing and magnitude of seedling emergence differed among study seasons (Table 1: cohort $P < 0.05$). Peak emergence for cohort 2000 was delayed to late summer (March), whereas for cohorts 2001 and 2002 emergence occurred mostly in early summer (January). Total seedling emergence of cohort 2000 was very low (mean ± SE, 1.1 ± 0.6 plants/plot). Emergence markedly increased for cohort 2001 (8.5 ± 3.5 plants/plot) and was intermediate for cohort 2002 (4.0 ± 2.5 plants/plot). The last cohort emerged from seed sown during the previous 2 years, indicating that *G. triacanthos* seeds remained viable in the soil for at least 1 year.

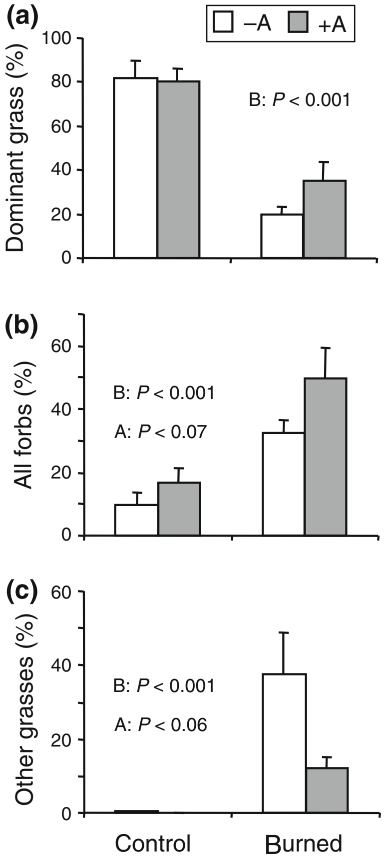

Fig. 2 Effects of burning (B) and simulated armadillo (A) disturbances on grassland community composition. *Bars* show mean (+ SE, $n = 5$) percent cover for **a** dominant tussock-grass, *Paspalum quadrifarium*, **b** all herbaceous forbs, and **c** other grasses, as measured 18 months after burning (December 2000). *P*-values are from two-way ANOVA

Disturbance by burning and armadillo-like diggings had significant but opposing effects on tree emergence (Table 1; Fig. 4a). Simulated armadillo disturbances exerted an overall negative impact on seedling numbers, which was stronger for cohorts 2001 and 2002 (both $P < 0.0001$) than for cohort 2000 ($P = 0.044$, not significant after Bonferroni's

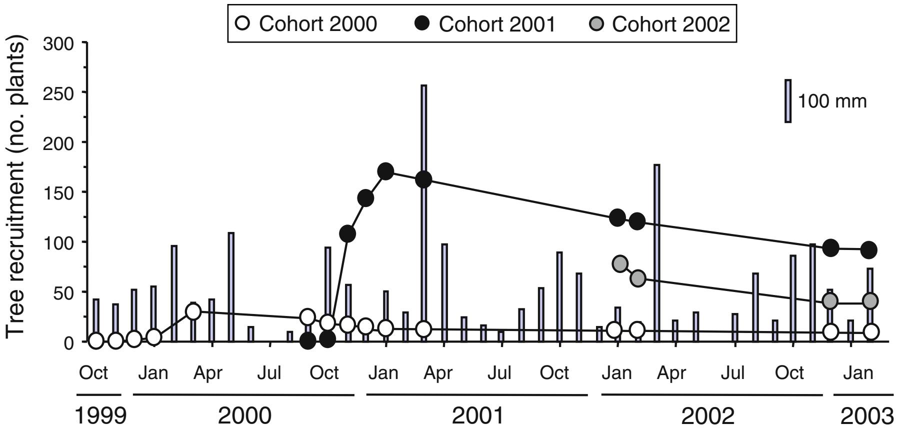

Fig. 3 Overall recruitment dynamics of the invasive tree *Gleditsia triacanthos* in a relict tussock grassland, Inland Pampa, Argentina. Data show total number of plants for three seedling cohorts (2000–2002), with plants pooled over all experimental plots (total area: 240 m^2). Cohort 2002 emerged 1 year after the last sowing event in December 2000. *Grey bars* show monthly precipitation (mm) for the study area

Table 1 Results of likelihood-ratio tests for the effects of disturbance regimes on tree seedling emergence and establishment in a tussock grassland relict

	df	Emergence		Establishment	
		χ^2	P	χ^2	P
Burning (B)	1	4.28	0.0385	7.29	0.0069
Armadillo (A)	1	40.43	0.0001	26.80	0.0001
Cohort (C)	2	7.56	0.0229	8.60	0.0136
B × A	1	10.98	0.0009	10.45	0.0012
B × C	2	4.78	0.0912	5.91	0.0521
A × C	2	9.91	0.0071	8.39	0.0151
B × A × C	2	5.38	0.0680	6.22	0.0446

Emergence represented peak seedling numbers for three cohorts (2000–2002). Establishment denoted the number of plants surviving at the end of the study (March 2003). Seedling count data were log-transformed and modelled assuming Gaussian errors (residual *df* = 48)

correction). In contrast, burning increased seedling emergence, but only in the absence of armadillo-like disturbances (burning × armadillo $P < 0.001$; Table 1; Fig. 4a). This pattern was qualitatively consistent, but differed in magnitude among study seasons (three-way interaction $P < 0.07$; Table 1), being highly significant for cohort 2001 ($P = 0.0003$) and non-significant for cohorts 2000 ($P = 0.52$) and 2002 ($P = 0.11$). Indeed, our burrowing regime precluded the emergence of cohorts 2001 and 2002 in burned plots (see Fig. 4a).

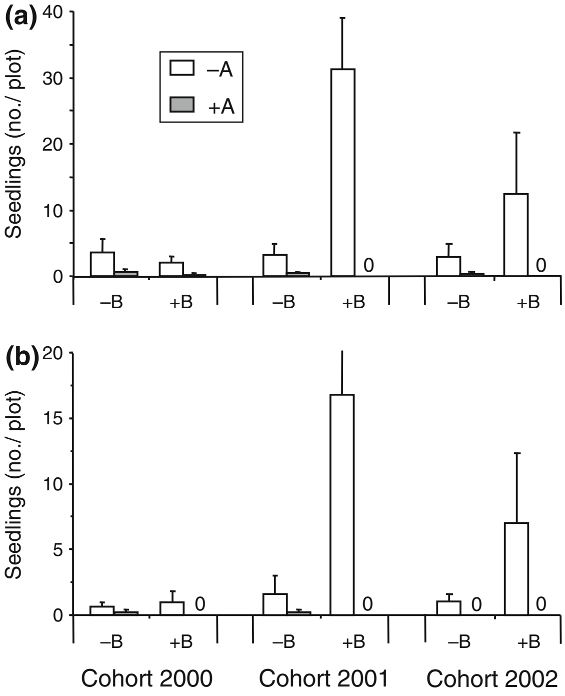

Fig. 4 Effects of burning and simulated armadillo burrows on **a** seedling emergence and **b** final establishment of *Gleditsia triacanthos* in a tussock grassland relict, Inland Pampa, Argentina. *Bars* show means + SE ($n = 5$) for three plant cohorts emerged in consecutive years (2000–2002). Establishment refers to plants found alive in March 2003. '0' denotes that no seedlings were found. Note the change of scale between panels. B: burning, A: armadillo. See Table 1 for statistical results

Tree seedling survival was lower for cohort 2000 than for cohorts 2001 and 2002 (29, 48 and 51%, respectively). However, we found no significant effect of disturbance treatments on survivorship for any of the three cohorts (log-linear tests: $\chi^2 < 2.83$, $P > 0.10$). Tree establishment at the end of the study largely reflected patterns generated during emergence (Fig. 4). Final recruitment depended on the cohort (Table 1), being much higher for cohort 2001 (4.7 ± 2.0 plants/plot) than for cohorts 2000 (0.5 ± 0.2 plants/plot) and 2002 (2.0 ± 1.4 plants/plot). Overall, this reflected the increased number of trees recruiting successfully in burned-only plots (Table 1; Fig. 4b). Disturbances remained a significant control of establishment from cohorts 2001 (burning × armadillo $P = 0.0014$) and 2002 (armadillo $P = 0.01$), but not cohort 2000 (armadillo $P = 0.078$). Noticeably, in the absence of disturbance, the study grassland did not manage to repel tree invasion (see Fig. 4b).

Disturbances and resource availability

Light penetration (PAR) beneath undisturbed vegetation was 12.4 ± 4.1%, while newly created armadillo burrows increased light availability to 85.4 ± 2.6% of ambient PAR. Burning significantly reduced soil water content and slightly increased soil available P (Table 2). Soil N availability (especially NO_3^-) was significantly increased on simulated armadillo burrows (Table 2). This effect was mostly evident in burned plots, yet the burning × armadillo interaction was only marginal ($P < 0.10$).

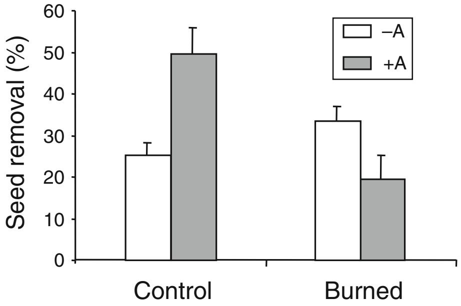

Fig. 5 Tree seed loss to rodent granivores under different disturbance regimes in a tussock grassland relict. *Bars* show percentage seed removal (mean + SE, $n = 5$), estimated from the difference in the number of intact seeds remaining inside and outside cage exclosures. A: simulated armadillo burrows

Tree seed loss to granivores

Small-mammal exclusion significantly reduced total seed loss (cage effect $F_{1,\ 118} = 49.95$, $P < 0.0001$). Seed loss to rodents averaged 31.1 ± 25.9% for the whole experiment. In addition, substantial amounts of tree seeds were lost to invertebrate consumers, including insects and slugs, which reduced seed survival at 45.3 ± 3.2% per plot. The date of experiment had no significant effect on seed removal patterns (date and date × treatment effects, $P > 0.10$). Burning and armadillo-like diggings strongly interacted in affecting seed removal by rodents ($F_{1,8} = 18.28, P = 0.003$). In unburned plots, tree seed loss was higher in simulated burrows than beneath intact canopy (Fig. 5), whereas in burned plots, seed removal did not significantly differ between armadillo and control microsites. Disturbances did not

Table 2 Effects of burning (B) and simulated armadillo (A) disturbances on soil water content, and soil available phosphorus and nitrogen concentrations

	Treatment				Two-way ANOVA		
	−A −B	+A −B	−A +B	+A +B	A	B	A × B
Water (%)	19.4 ± 1.7	18.2 ± 1.1	14.6 ± 2.7	11.2 ± 1.0	1.61	10.69**	0.36
PO_4^{2-} (μg/g)	24.9 ± 2.0	27.3 ± 1.9	27.9 ± 3.9	34.2 ± 2.7	2.52	3.34†	0.50
NO_3^- (μg/g)	0.59 ± 0.16	1.35 ± 0.53	0.27 ± 0.10	2.11 ± 0.65	13.27**	0.12	2.98
NH_4^+ (μg/g)	3.75 ± 0.83	4.79 ± 1.66	3.96 ± 1.03	10.24 ± 1.48	6.88*	4.13†	3.53†
N_{min} (μg/g)	4.35 ± 0.85	6.13 ± 2.06	4.23 ± 1.13	12.35 ± 2.24	8.85**	3.35†	3.61†

N_{min} total mineral nitrogen. Values are means ± SE ($n = 5$) for composite soil samples (December 2000). Also shown are results (*F*-ratios) of two-way ANOVAs ($df = 1, 16$)

** $P < 0.01$, * $P < 0.05$, † $P < 0.10$

influence seed predation by invertebrates (all P values >0.10, data not shown).

Discussion

Common wisdom suggests that disturbances generally promote invasion by exotic plants (Williamson 1996; D'Antonio et al. 1999; Lockwood et al. 2007). In this study, a relictual fragment of pampean grassland was subjected to contrasting disturbance regimes under high propagule pressure from an invasive woody species. We found that disturbances affected grassland invasibility, although the magnitude and direction of effects depended on the plant cohort and the interaction between disturbances. First, disturbance effects on tree establishment were mostly apparent during a 2-year wet period. Second, burning and armadillo-like diggings exerted antagonistic effects on tree recruitment. These findings support the view that, rather than having readily predictable consequences on invasion, disturbances interact with environmental context in creating a dynamic patchwork of recruitment opportunities for exotic plants (Davis et al. 2000; Shea and Cheeson 2002). Such contingencies may influence the pattern and rate of woody species encroachment on grass-dominated ecosystems (Scholes and Archer 1997; Sankaran et al. 2006; Bond 2008).

Temporal variation in tree invasion success

The magnitude of invasion, as reflected by tree establishment success, was contingent on the focal seedling cohort (Figs 3, 4). Environmental conditions during seed germination were clearly important for *G. triacanthos*, since initial differences in emergence between cohorts largely determined final recruitment patterns. Hence, seedling emergence, not survival, represented the main demographic bottleneck for tree invasion in this tussock grassland (cf. Bond 2008). We focussed on the early establishment phase of the invasion process (Levine et al. 2004). Simulation models show that the generation of seedling banks is a major determinant of population success in *G. triacanthos* (Cannas et al. 2003). Seedling recruitment may thus play a key role in the long-term woody encroachment of remnant pampean grasslands (Facelli and León 1986; Mazía et al. 2001; see also Scholes and Archer 1997).

Inter-annual differences in tree emergence suggested that extrinsic factors such as precipitation and seed availability were probably important in affecting the chances of invasion (Crawley 1989; Thomsen et al. 2006; Boulant et al. 2008). Weather conditions differed between sowing seasons. Germination of hard-coated *G. triacanthos* seeds is known to be primarily controlled by soil moisture (Burton and Bazzaz 1991). Here, below-average rainfall in the first spring was accompanied by the reduced and delayed emergence of cohort 2000, whereas high rainfall levels in the ensuing years corresponded with the enhanced emergence of two other cohorts (Figs 3, 4). Short-term decreases in water availability may also have lasting effects on invasibility through increased competition from established plants on emerging seedlings (O'Connor 1995; Davis et al. 1998; Davis and Pelsor 2001). Nevertheless, recruitment of cohorts 2001 and 2002 was limited to burned-only plots, suggesting a protracted effect of fire that interacted with rainfall levels in controlling tree emergence dynamics. Reduced litter cover in burned grassland may expose seeds to high radiation and temperature fluctuations, triggering germination under adequate moisture conditions (Burton and Bazzaz 1991; Grime 2001).

On the other hand, we cannot reject propagule pressure as a factor limiting recruitment of the first seedling cohort. Our seed addition protocol meant that despite substantial loss to resident granivores (up to 75% of sown seeds) propagules became increasingly available, until sowing was discontinued in late spring 2000. It is therefore possible that seed availability could partly account for the observed differences in seedling recruitment among years. This would be consistent with the lowered density of cohort 2002, which emerged during a wet season but only from remnant seeds added the previous year (see Fig. 3). These results emphasise the importance of interactions among stochastic factors including propagule arrival, environmental fluctuation and large-scale disturbances in driving temporal patterns of plant invasion (Crawley 1989; Simberloff 2009).

Disturbance interaction and tree invasion success

Tree seedling invasion was dependent on the applied disturbance regime. Initial burning increased community invasibility by *G. triacanthos*, but only in the absence of frequent disturbance by simulated armadillo diggings, because artificial diggings virtually precluded tree recruitment (Fig. 4). These patterns show that interactions between disturbance agents may alter predictions from simple disturbance–invasibility models (see also Alpert et al. 2000; Chaneton et al. 2002). Burning and animal diggings are common disturbances in grasslands (Hobbs and Huenneke 1992; D'Antonio et al. 1999). Burrowing armadillos are major constituents of the native fauna of the Pampas (Soriano 1992; Bilenca and Miñarro 2004), being widely distributed across relictual and old-field grasslands (Machera 2006). In contrast, fire occurs mostly as a human-made disturbance, which is sporadically used to improve forage quality for domestic grazing (Ghersa and León 1999; Laterra et al. 2003). Cattle movement may facilitate dispersal of *G. triacanthos* seeds from distant woodland lots to remnant grassland fragments from which the species was absent (Marco and Páez 2000; Mazía et al. 2001).

When applied alone, burning increased seedling recruitment from cohorts emerged in 2001–2002. Fire drastically altered the grassland structure by suppressing the dominant tussock-grass species (Fig. 2). Total aboveground biomass showed little post-fire recovery, even after two growing seasons, and despite widespread invasion by exotic herbs. However, burning did not affect establishment of the first seedling cohort (2000), which could reflect seed-limited recruitment as discussed above. Most likely, it appears that microsite conditions in burned plots were inadequate for tree emergence during the dry spring of 1999, as fire itself decreased soil water content (see Table 2). The remarkable increase in tree recruitment during wetter years suggested that exotic forbs colonising the burned grassland were 'weak' competitors (Ortega and Pearson 2005), unable to displace woody seedlings under higher water availability (Davis et al. 1998). In contrast, the undisturbed grassland was more resistant to invasion, probably because tree emergence was inhibited by the presence of a thick litter layer (Xiong and Nilsson 1999) and/or seed predation by resident granivores (Murillo et al. 2007).

Small-scale animal disturbances have been found to either promote or suppress exotic species (Hobbs and Huenneke 1992; D'Antonio 1993; Kotanen 1997). Soil disturbances by burrowing mammals create gaps within closed canopies and may also alter soil resource availability (D'Antonio et al. 1999). In the study system, armadillo-like diggings did not kill established grasses but removed the litter and turned over the soil in-between tussocks. As a result, small-scale disturbances increased light levels and soil nitrogen availability (Table 2), but had a small impact on community composition (Fig. 2), as artificial burrows were rapidly filled by resident grasses. Against expectation, rather than creating safer microsites for establishment, our digging regime suppressed seedling emergence, which indicated that small-scale disturbances offset invasion by reducing tree seed survival and/or germination (Fig. 4a). Indeed, newly emerged tree seedlings were very rarely found on armadillo-like mounds. This was most obvious in burned plots, which were otherwise highly susceptible to *G. triacanthos* invasion. Thus, the impact of a single, large-scale perturbation promoting grassland invasion by exotic trees was overridden by a regime of frequent, small-scale disturbances. These results counter those from other systems in which soil disturbances increased woody seedling establishment within a grass-dominated matrix (D'Antonio et al. 1999; Setterfield 2002; Dickie et al. 2007).

The negative impact of armadillo diggings on tree emergence may have several non-exclusive explanations. First, soil mounds may dry up faster than closed-canopy areas, and thus *G. triacanthos* seeds landing on disturbed patches would encounter unfavourable microclimate for germination (Burton and Bazzaz 1991). Yet this idea is weakly supported by soil water measurements taken in spring 2000 (Table 2). Second, tree seeds might become buried by soil removed from artificial diggings. While we have no direct evidence for this mechanism, we certainly cannot dismissed it. Third, disturbances associated with armadillo burrows may influence the foraging behaviour of resident consumers (D'Antonio et al. 1999). Consumers may provide biotic resistance to plant invasions (e.g. MacDougall and Wilson 2007; Nuñez et al. 2008), and their role can be modified by both large- and small-scale disturbances (D'Antonio et al. 1999). We hypothesise that simulated armadillo gaps may have increased tree seed/seedling predation, perhaps by enhancing their visibility to consumers. Our seed removal trials lend partial support to this idea, as seed loss to rodents increased within artificial diggings created in unburned plots (Fig. 5). Granivorous rodents have been found to reduce herbaceous plant invasion in other tussock Pampa grasslands (Murillo et al. 2007). While the exact mechanism remains unknown, our results suggest that armadillo burrows may enhance community resistance to tree invasion in native tussock grassland relicts.

Our findings are compatible with the notion that maintaining a dense herbaceous cover helps to prevent woody species invasions in grasslands and savannas (Scholes and Archer 1997; Davis et al. 1998; De Blois et al. 2004; Dickie et al. 2007). They also point out that small-scale animal disturbances may act as barriers to tree recruitment, even in the context of large-scale perturbations such as burning (cf. D'Antonio 1993). We nevertheless found that the undisturbed tussock grassland was not completely immune to invasion by *G. triacanthos* (Fig. 4). Mechanisms of invasion resistance can be overwhelmed by high propagule pressures from invaders with the 'right' traits (Williamson 1996; von Holle and Simberloff 2005). Large and frequent seed dispersal events, such as those simulated by our study, may counteract the negative impacts of demographic and environmental stochasticity on the likelihood of initial population establishment (Simberloff 2009).

Conclusions

We have shown that, after adjusting for propagule pressure, the disturbance regime was a major determinant of tree invasion success in native tussock grassland. Yet, invasibility patterns reflected unexpected interactions between anthropogenic and natural disturbance agents, which emerged in the context of stochastic variation in environmental conditions. Under modern scenarios of high propagule pressure from exotic species (Williamson 1996; Simberloff 2009), human-induced alterations of disturbance regimes increase the risk of invasion by relaxing mechanisms of biotic resistance dependent on resident competitors and consumers (Elton 1958; D'Antonio et al. 1999; Alpert et al. 2000). In the Inland Pampas, native grasslands have been increasingly confined to small relict fragments and corridors. While undisturbed tussock-grass communities may be relatively resistant to invasion (Laterra et al. 2003; Murillo et al. 2007), we found that burning greatly increased community invasibility by exotic trees. A state-shift towards woody-dominated systems may be inevitable (Petraitis and Latham 1999), if seed sources of introduced tree species continue to expand across pampean landscapes, and relicts of native grassland are not adequately protected by law (Bilenca and Miñarro 2004). Interestingly, our results suggested that frequent soil disturbances by burrowing armadillos may contribute a significant source of resistance to tree expansion on remnant grasslands. Management of tussock grassland habitats for native fauna may thus feedback on their own conservation in the face of ongoing exotic invasions and land-use changes.

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