

Invasive exotic grasses and seed arrival limit native species establishment in an old-field grassland succession

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Received: 16 January 2012 / Accepted: 9 May 2012 / Published online: 26 May 2012
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Abstract Plant communities developing in abandoned semi-natural areas are being increasingly dominated by invasive exotic species. How these ‘novel residents’ affect re-colonisation by native perennial species, a process generally assumed to be seed limited, remains little explored. We examined the relative roles of dominant exotic grasses and seed dispersal in limiting the richness and abundance of native perennial grasses in an old-field grassland community. We also tested whether native grass recruitment depended on the identity of resident exotic species. A seed addition, single-pulse removal experiment was established in a 20 year old field in the Inland Pampa of Argentina. Seeds of seven native perennial grasses from a nearby relict grassland were sown into intact and disturbed patches dominated by one of four exotic grasses. Species richness and biomass were measured after 2 years from sowing. Seed addition alone had little effect on native grass richness or biomass, with only one sown grass establishing in intact patches. Native grasses successfully colonised disturbed patches

dominated by the exotics *Lolium multiflorum*, *Cynodon dactylon* or *Sorghum halepense*. In contrast, patches dominated by *Festuca arundinacea* repelled sown native grasses, regardless of disturbance treatment. Seed addition increased total plant richness in both disturbed and intact patches but did not affect total aboveground biomass. Our results show that recovery of native grasses during old-field succession is hierarchically constrained by seed arrival and site pre-emption by exotic grasses. Thus, re-establishment of native grass assemblages may only occur at the expense of displacing exotic resident plants. This highlights the importance of niche-limited species assembly in novel, native/exotic plant communities.

Keywords Dispersal limitation · Novel ecosystems · Invasion impact · Richness · Removal experiment · Seed addition

Introduction

Successional communities developing on degraded, abandoned semi-natural areas often become dominated by exotic plants (Simberloff 2010), while native species may be slow to recover or otherwise fail to establish altogether (Meiners et al. 2002; Kulmatisky 2006; Baer et al. 2009; Tognetti et al. 2010). In highly fragmented landscapes, native species recovery would be limited by dispersal from remnant seed sources (Seabloom et al. 2003), but could also reflect priority

Electronic supplementary material The online version of this article (doi:10.1007/s10530-012-0249-2) contains supplementary material, which is available to authorized users.

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effects from invasive exotic plants (Corbin and D'Antonio 2004; MacDougall and Turkington 2005; Standish et al. 2007). Exotics can be regarded as present-day drivers of community dynamics, despite not being the original cause of native diversity declines in the past (cf. MacDougall and Turkington 2005). Intriguingly, whereas effects of native plants on exotic species success have been tested in many systems (Emmery and Gross 2006; Daneshgar and Jose 2009), few experiments have examined the impact of different exotic invaders on native species recruitment (Levine et al. 2003; Vilá et al. 2011). The specific identity of dominant exotic residents may interact with seed availability in altering the chances that native species recolonise historically disturbed sites (Emery and Gross 2006, 2007; Cramer et al. 2008; Seabloom 2011).

Plant recruitment depends on seed and microsite availability (Eriksson and Ehrlén 1992). The relative importance of both these factors changes with community structure, seed size and disturbance regime, among other factors (Turnbull et al. 2000; Clark et al. 2007; Myers and Harms 2009). Seed availability is controlled by dispersal rate and seed production, and by local mortality processes including seed predation (Maron and Gardner 2000). Sowing experiments that focus on species present in a local community allow one to evaluate the extent of seed limitation of recruitment, whereas introductions help to discern if a given species is locally absent because of dispersal limitation or lack of adequate niche conditions (Pacala and Rees 1998; Münzbergová and Herben 2005; Funk et al. 2008). Microsite availability is related to local abiotic conditions and can be also strongly determined by plant diversity, composition, and productivity (Tilman 1993; Turnbull et al. 2000; Fargione et al. 2003; Clark et al. 2007). Moreover, microsite limitation is modulated by disturbances (Zobel et al. 2000; Foster et al. 2004). In productive habitats, disturbance generally enhances seedling recruitment, but may also exacerbate seed limitation by increasing the number of suitable microsites (Eriksson and Ehrlén 1992; Clark et al. 2007).

The effect of established exotic species on native plant colonisation will likely depend on their ability to monopolise resources and inhibit recruitment (Connell and Slatyer 1977; Emery and Gross 2006). Many exotic plants exhibit traits that make them strong competitors in resource-rich environments, including

large size, rapid growth rate, extensive lateral spread, copious litter production, and allelopathy (Grime 2001; Daehler 2003; Hierro and Callaway 2003). Invasive exotics may also differ in their ability to withstand disturbance. Patches dominated by long-lived, disturbance resistant exotics would lessen recruitment opportunities for arriving propagules (Symstad et al. 1998; Crawley et al. 1999), thus having a large impact on native species recruitment. We therefore expect the identity of exotic residents to play a critical influence on the establishment of dispersal-limited native species in old fields.

Invasive exotic plants may exert significant impacts on biodiversity, with potential consequences for ecosystem-level attributes (Levine et al. 2003; Vilá et al. 2011). Such effects might be alleviated if removal of invasive species reduces seed or microsite limitations to native recruitment (MacDougall and Turkington 2005). Seed addition to grasslands has been found to increase both species richness (Zobel et al. 2000; Dickson and Foster 2008; Myers and Harms 2009) and primary productivity (Stein et al. 2008), especially when disturbances weaken competition from resident plants (Foster et al. 2004; Myers and Harms 2011). Introducing seed-limited native species into exotic-dominated communities might increase diversity, and perhaps biomass production through niche complementarity or selection effects (Loreau et al. 2001; Wilsey et al. 2009), or may just compensate for exotics being displaced by targeted removals.

In this study, we evaluate the relative roles of invasive exotic species and seed arrival in limiting recruitment of native perennial grasses in abandoned agricultural fields of the Inland Pampa, Argentina. Old-field grasslands in the Pampas comprise a community mosaic dominated by annual and perennial exotic grasses (Tognetti et al. 2010). The scarcity of native grasses in old fields has been mostly attributed to propagule limitation associated with isolation and low seed arrival produced by landscape fragmentation (D'Angela et al. 1988; Omacini et al. 1995).

We hypothesised that native grass establishment is limited by reduced seed dispersal and by interference from exotic grasses in a hierarchical fashion (cf. Funk et al. 2008). First, native pampean grasses would be limited by propagule arrival, so that seed addition should increase native plant richness and biomass in old fields. Second, native grass seedlings would be

excluded by asymmetric competition from established exotic grasses. Thus, seed sowing should have a greater positive effect on native grass establishment in disturbed patches (i.e., seed addition \times exotic removal). Third, the extent of recruitment inhibition would depend on the identity of resident exotics. We expected colonisation success of sown native grasses to decrease across patches dominated by exotics of increasing competitive ability. To test these ideas, we performed a seed addition, removal experiment in a 20 year-old successional grassland. A mix of native perennial grasses was sown into four different exotic patch types, which were left intact or disturbed. Species richness and standing biomass were measured after 2 years to determine how re-introducing native grasses affected community attributes in the short term.

Methods

Study site

The experiment was conducted in a farm owned by the University of Buenos Aires near the village of Hortensia, some 400 km west of Buenos Aires city, Argentina (35° 53' S; 61° 12' W). The area is located on the eastern side of the Inland Pampa district (Soriano 1992). The climate is temperate sub-humid, with mean temperatures of 23.4 °C in January and 8.2 °C in July. Mean annual precipitation is 1,090 mm (1976–2009). Soils are well-drained, sandy-loam Typic Hapludols, with 2.5 % organic matter. The experimental site was located within a 5-ha, old-field grassland enclosure, which had remained free of agriculture and grazing for 20 years. This enclosure represented an island of semi-natural vegetation embedded in an extensive agricultural matrix. Old-field succession in this system has been described elsewhere (Omacini et al. 1995; Tognetti et al. 2010). The vegetation was representative of mid-seral communities dominated by the exotic grasses *Festuca arundinacea*, *Cynodon dactylon*, *Sorghum halepense* and *Lolium multiflorum* (hereafter referred to by genus), with a few native ruderals including *Bromus catharticus* and *Deyeuxia viridiflavescens*, and exotic tall forbs such as *Carduus acanthoides* and *Conium maculatum* (nomenclature, Cabrera and Zardini 1978). The closest remnant of native grassland stretches along an abandoned railway track 5 km west of the

study site. Native grasses are also scattered across landscape “refuges” such as fencerows and grazed fields (Poggio et al. 2010; Burkart et al. 2011).

Experimental design and data collection

The experiment was initiated in 2006 and harvested in 2008. The first 2 years had nearly average rainfall conditions for the area (2006: 1,038 mm, 2007: 945 mm); however, 2008 was a rather dry year (total rainfall: 650 mm). In May 2006, we selected four patch types dominated by *Lolium*, *Cynodon*, *Sorghum* or *Festuca* within a topographically homogeneous area of 1.5 ha. Small depressions and woody thickets (*Ulmus pumila*) were avoided. Only patches unambiguously dominated by one of the four focal species were considered (i.e., >90 % cover for *Cynodon*, *Sorghum* or *Festuca*, and >60 % cover for *Lolium*). Each patch type was replicated in four different locations (16 patches in total). To reduce edge effects and ensure independence, patches were at least 16 m² in size and were distanced by 20 m from each other.

In order to rank the potential interference of these exotic patches on native grass establishment, we calculated a competitive index for each focal exotic grass based on Grime’s CSR model (Grime 1974; Hodgson et al. 1999). This index synthesises important functional traits as leaf canopy height, lateral vegetative spread and litter production, and ranges between 1 and 10, from low to high competitive species. The resulting competitive index increased in the order *Lolium* < *Cynodon* < *Sorghum* < *Festuca* (for details, see Online Resource 1). *Lolium* is a fast-growing, annual bunchgrass that produces a thick litter layer, and often occurs in mixed patches with native grasses *B. catharticus* and *D. viridiflavescens*. The other three exotics are perennials with different capacity for lateral spread, which generates nearly monospecific patches. *Cynodon* is a stoloniferous, prostrate species, with extensive lateral clonal growth. *Sorghum* is a tall rhizomatous weed species, while *Festuca* is a tall-tussock grass with short rhizomes forming compact patches. *Lolium* and *Festuca* are cool-season (C₃) whereas *Cynodon* and *Sorghum* are warm-season (C₄) grasses. All four are known to be allelopathic (Gibson 2009), which in *Lolium* and *Festuca* may reflect symbiosis with fungal endophytes (Cheplick and Faeth 2009). All four species are widespread exotic invaders in other grasslands as well (e.g. Wilsey et al. 2011).

The full experiment was a three-way factorial design with four patch types (i.e., exotic species identity), two disturbance levels (with or without canopy removal) and two seed addition levels (with or without). There were four replicates per treatment combination, yielding 64 experimental units in total. Sowing and removal treatments were nested within patch types in a split-plot design without blocks (Steel and Torrie 1980); treatments were applied to four 0.5×0.5 m subplots delimited near the centre of each experimental patch (main plot). A single, pulse-removal disturbance (Bender et al. 1984) was applied only once at the beginning of the experiment (late autumn 2006) to half of the subplots within each patch. The disturbance consisted in clipping and removing all standing vegetation to ground level and then raking the topsoil. Our removal treatment simulated management actions aiming to control exotic invaders as well as small-scale natural disturbances (e.g. grazing, burrowing). We decided not to use herbicides to preclude any residual effects on soil biota (Potthoff et al. 2009). Hence, differences in the regrowth ability and phenology of focal exotic species likely influenced native grass establishment in our removal patches.

Half of the subplots were sown with seven native perennial grasses comprising a range of seed sizes and functional types (Table 1). All selected species occur in relictual and grazed grasslands throughout the region (Burkart et al. 2011). However, only two of them have been ever recorded during old-field succession in the study area (Table 1). In each designated sub-plot, we added a total of 8,000 viable seeds m^{-2} , evenly distributed among sown species. This sowing density

was intended to minimise seed limitation and was in the range of propagule pressures reported for other grassland studies (e.g., Foster and Tilman 2003; Foster et al. 2004). Seeds were collected from native grassland remnants located within 10 km of the experimental site, and were air-dried and stored at 5 °C.

Prior to treatment application, exotic patches were characterised by plant cover, species richness and percent light interception. Plant cover was visually estimated for all species rooted within a 2×2 m quadrat placed at the centre of each patch [according to a modified Braun-Blanquet 5 % increment scale (see Tognetti et al. 2010)]. Richness denoted the number of plant species per 4 m^2 . Light interception was measured using a 1-m long ceptometer (Cavadevices[®], Buenos Aires, Argentina). Photosynthetic photon flux density (PPFD $\mu mol m^{-2} seg^{-1}$) was measured above the leaf canopy and below the vegetation at two crossed positions per patch and then averaged for analysis. In late December 2008, all aboveground biomass was harvested from a 0.2×0.4 m quadrat placed at the centre of each sub-plot. Plants were clipped to ground level and separated into live and dead parts; live biomass was sorted by species, oven-dried at 60 °C during 72 h and weighed. Species number and biomass were obtained for sown native grasses, unsown native grasses, all exotic grasses and all forbs.

Data analysis

To summarise patterns of treatment effects on community composition, we performed non-metric multidimensional scaling (NMDS) ordination based on

Table 1 Native grass species used in the seed addition experiment

Species name	FG	Seed mass (mg)	GP (%)	Freq (%)	Cover (%)	+S (%)	–S (%)
<i>Briza subaristata</i>	C3	0.462	90	10	0.6	31.3	0
<i>Melica brasiliana</i>	C3	3.528	92	0	0	37.5	3.0
<i>Paspalum quadrifarium</i>	C4	0.299	3	0	0	9.4	0
<i>Piptochaetium bicolor</i>	C3	5.766	90	0	0	0	0
<i>Piptochaetium montevidense</i>	C3	0.852	58	0	0	9.4	0
<i>Schizachirium scoparia</i>	C4	1.088	88	0.8	0.5	*	0
<i>Stipa neesiana</i>	C3	1.034	66	0	0	9.4	0

All species were typical of pristine pampean grasslands, but were sparse or absent in old-field communities. Data show species frequency (Freq) and mean cover, as recorded for 20 years of old-field succession in the study site. Also shown is each species occurrence (percent of subplots in which the species was found at final harvest) in experimentally sown (+S) and unsown (–S) subplots, pooling over intact and disturbed plots. FG functional group; GP germination potential under laboratory conditions. * A species present but not harvested in a given treatment

Table 2 Aboveground biomass (g m^{-2}) of native and exotic grass species in seed addition plots within each of four patch types in a 20-year old field in the Inland Pampa, Argentina

Species	Lolium patch		Cynodon patch		Sorghum patch		Festuca patch	
	Intact	Removed	Intact	Removed	Intact	Removed	Intact	Removed
Sown native grasses								
<i>B. subaristata</i>	0.2 ± 0.2	210.2 ± 96.9		207.4 ± 136.1		34.7 ± 20.9		
<i>M. brasiliana</i>	8.4 ± 8.4	19.8 ± 18.0	11.4 ± 11.4	135.8 ± 78.9	31.1 ± 31.1	208.3 ± 136.7		
<i>P. quadrifarium</i>		36.1 ± 32.8		1.0 ± 1.0				
<i>P. montevidense</i>		0.6 ± 0.4				0.1 ± 0.1		
<i>S. neesiana</i>		2.2 ± 2.2		5.2 ± 3.8				
Unshown native grasses								
<i>Leersia hexandra</i>	16.4 ± 15.5	10.7 ± 10.7	9.6 ± 4.5	1.1 ± 0.7	6.1 ± 6.1	1.2 ± 1.2	3.8 ± 3.8	2.0 ± 1.4
<i>Bromus catharticus</i>	8.2 ± 6.6	6.7 ± 5.4	42.3 ± 34.9	42.8 ± 42.3	23.9 ± 14.7	4.5 ± 4.5		
<i>Deyeuxia viridiflavescens</i>		15.4 ± 12.0			1.3 ± 1.3	9.8 ± 7.2		
Exotic grasses								
<i>L. multiflorum</i>	25.1 ± 21.8	7.3 ± 7.3						
<i>C. dactylon</i>	28.9 ± 12.8	28.9 ± 16.8	47.6 ± 18.1	34.8 ± 9.8	50.1 ± 15.1	58.2 ± 19.0	8.0 ± 7.7	10.6 ± 7.6
<i>S. halepense</i>	20.3 ± 11.2	44.4 ± 23.9			53.5 ± 37.3	19.7 ± 12.0		68.0 ± 68.0
<i>F. arundinacea</i>	43.6 ± 43.6	100.8 ± 66.0	1.1 ± 1.1	181.9 ± 107.7		51.7 ± 51.7	1067.1 ± 515.0	141.1 ± 116.6

Patch types were defined by dominant exotic grass. Data show means ±SE (n = 4) for intact and exotic removal sub-plots after 2 years of experiment

individual species biomass at the end of the experiment (McCune and Mefford 1999). We used the Bray-Curtis dissimilarity index as distance measure. To optimise the analysis, starting coordinates were provided by sample scores derived from Detrended Correspondence Analysis of the same dataset (Minchin 1987). A two-dimensional configuration (stress = 0.22) was retained as an adequate portrayal of the distance structure in the data, after running a Monte Carlo test with 1,000 randomisations (McCune and Mefford 1999). Treatment effects on total and species group richness and biomass were evaluated using split-plot ANOVA models without blocks (Steel and Torrie 1980). Patch type was the main plot treatment, with seed sowing and exotic removals as fully crossed treatments at the sub-plot level. The patch type (exotic species identity) effect was tested against the random effect of plot (nested within patch). Data were log-transformed to meet assumptions of ANOVA. Tukey's honest significant difference (HSD) tests were performed to separate treatment means at $P < 0.05$. All analyses were implemented using Infostat software (Di Rienzo et al. 2011).

Results

Total aboveground and standing dead cover did not differ among the four patch types at the beginning of the experiment (both $F_{3, 12} < 1.7$ and $P > 0.10$). Species richness was lower in *Festuca* patches (2.5 spp) than in *Sorghum* (6.4 spp), *Cynodon* (6.7 spp) or *Lolium* (7.4 spp) dominated patches ($F_{3, 12} = 6.19$, $P < 0.001$; Tukey's HSD = 2.1 spp). Light interception was highest in *Festuca* patches (99.5 % of incident radiation), intermediate in *Cynodon* (97.6 %) and *Sorghum* (97.3 %) patches, and lowest in *Lolium* patches (90.2 %) ($F_{3, 12} = 21.6$, $P < 0.001$; Tukey's HSD = 0.4 %).

Two years after sowing, five of the seven sown grasses had established in at least one plot (Table 1). *Melica brasiliensis*, *Briza subaristata* and *Paspalum quadrifarium* accrued a sizeable amount of biomass (Table 2), reached reproductive stage and produced copious seeds in several plots (personal observation). *M. brasiliensis* also established in one unsown plot. *Piptochaetium montevidense* and *Stipa neesiana* occurred in just a few sown plots (Tables 1, 2), but did not flower during the experiment.

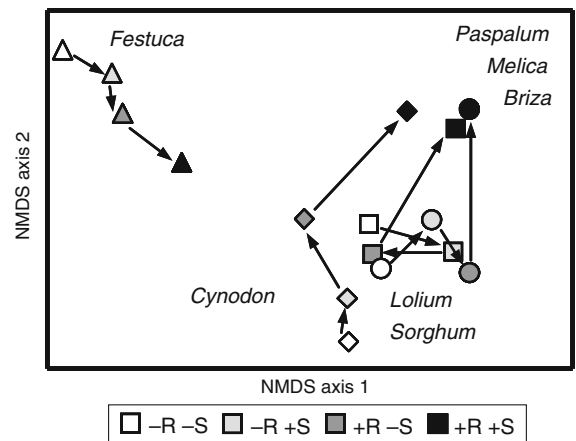


Fig. 1 Non-metric multidimensional scaling ordination showing effects of native grass addition and pulsed plant removal on exotic-dominated patches in an old-field community. Symbols denote centroids ($n = 4$) for each of four patch types, after 2 years of study. Patches were initially dominated by *L. multiflorum* (circles), *C. dactylon* (diamonds), *S. halepense* (squares) or *F. arundinacea* (triangles). Arrows connect treatments within patch types. Treatments: +S = sown, -S = unsown, -R = control, +R = removal. The relative position of major grass species in ordination space is shown after weighed averaging of individual species biomass along each axis

Seed addition and exotic plant removals jointly altered overall community composition in *Lolium*, *Cynodon* and *Sorghum* patches, mainly as a consequence of native grass establishment in sown + removal plots (Table 2; Fig. 1). In contrast, *Festuca* patches were little affected by the treatments and, when disturbed, slightly resembled *Cynodon* and *Sorghum* controls. Mean Bray-Curtis distances between control and sown + removal plots ranged 0.22–0.28 for *Lolium*, *Cynodon* and *Sorghum* patches, but only 0.08 for *Festuca* patches (Tukey test $P < 0.05$, after ANOVA; Fig. 1). Adding native grass seeds to intact patches did not substantially modify community composition (mean Bray-Curtis distance, control vs. sown plots = 0.12).

Seed addition, plant removal and patch type significantly interacted in affecting the number of established sown grasses (Table 3; Fig. 2a, b). Sowing had no consistent effect on species richness in intact patches, but increased native grass richness in *Lolium*, *Cynodon* and *Sorghum* removal patches. Conversely, seed addition did not influence richness in *Festuca* dominated patches, where none of the sown grasses managed to colonise, irrespective of disturbance (Fig. 2b). Richness of unsown native grasses, exotic

Table 3 Results of split-plot ANOVA (F -values) for the effects of patch type (P), seed addition (S) and exotic species removal (R) on plant species richness and live aboveground biomass

Source	$d.f.$	Species richness					Aboveground biomass				
		Sown native grasses	Unsown native grasses	Exotic grasses	All forbs	All species	Sown native grasses	Unsown native grasses	Exotic grasses	All forbs	All species
Patch type	3	5.8*	2.1	10.3*	3.4	13.5 ***	3.6*	1.83	2.2	4.8*	1.5
Sowing	1	59.6***	2.9	2.9	0.2	5.5 *	55.6***	5.1*	0.1	0.6	1.0
Removal	1	32.0***	0.7	0.2	0.8	8.9 **	23.0***	0.4	3.0	0.3	0.2
P × S	3	9.4***	0.6	0.5	0.1	0.9	6.5**	1.5	1.5	0.6	1.0
P × R	3	5.2**	1.1	2.1	0.3	0.3	3.2*	1.0	3.9*	0.1	4.8**
S × R	1	38.1***	0.7	0.7	0.8	0.3	28.1***	1.6	0.1	0.1	0.9
P × S × R	3	4.9**	0.3	0.7	0.9	0.4	3.2*	1.0	1.8	0.3	2.3

Sowing and removal treatments were nested within patch type. Error term $d.f.$ = 12 and 36, for the main plot and sub-plot effects, respectively

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

grasses and all forbs were significantly affected neither by native grass sowing nor by patch removals (Table 3; Fig. 2c–h). Exotic grass richness varied with patch type, being highest in *Lolium* dominated patches (Fig. 2e, f).

Seed sowing alone had little effect on native grass establishment, as only *M. brasiliiana* produced any substantial biomass in some intact patches (Table 2; Fig. 3a). However, seed addition significantly increased biomass of sown native grasses in removal plots, except for *Festuca* dominated patches (Table 3; Fig. 3a, b). Successful introduction of native grasses on disturbed plots was largely due to *B. subaristata* establishing in *Lolium* and *Cynodon* patches, and to *M. brasiliiana* in *Cynodon* and *Sorghum* patches (Table 2). Also, seed sowing and plant removal allowed *P. quadrifarium* to establish in *Lolium* patches, while *S. neesiana* and *P. montevidense* occurred with low biomass in different patches (see Table 2). On average, addition of these native grasses slightly reduced biomass of unsown native grasses, irrespective of treatment (Table 3; Fig. 3c, d). Removals had variable, species-specific effects on the unsown native grasses, which resulted in an overall non significant effect on this plant group (Table 2).

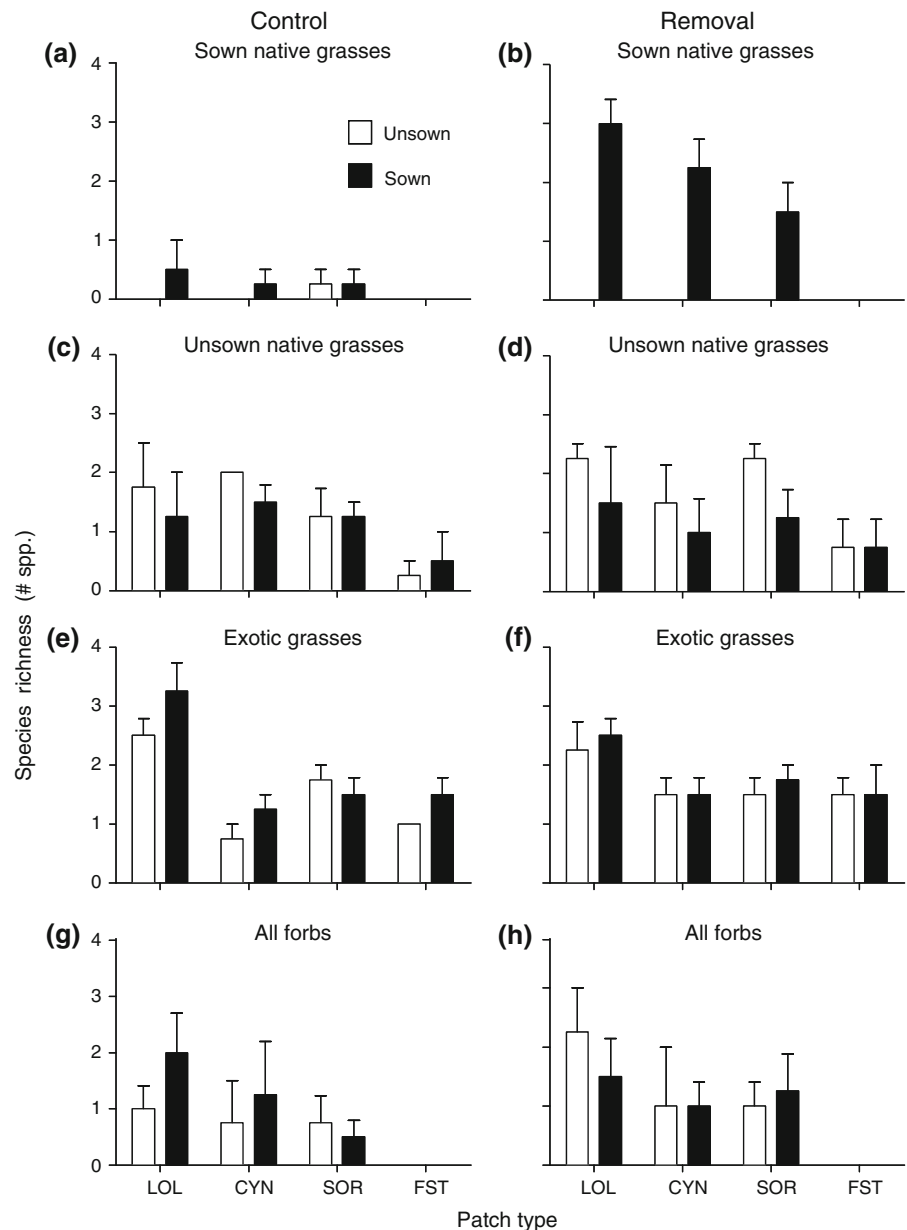
On the other hand, native grass sowing did not alter exotic grass biomass (Table 3). Removals were effective at reducing biomass of dominant exotic grasses according with patch type (Table 2). Further, at harvest, early removals had decreased total exotic grass biomass in *Lolium* and *Festuca* patches, whereas

exotic biomass increased in *Cynodon* removal patches (Table 3; Fig. 3e, f). The latter effect was related to the expansion of *Festuca* onto disturbed *Cynodon* patches (see Table 2). Lastly, forb species biomass was highest in *Lolium* and lowest in *Festuca* patches (Tukey's test $P < 0.05$), but was not influenced by native grass addition or plant removals (Table 3; Fig. 3g, h).

Overall, total plant richness was slightly, but significantly, increased by both seed addition (23 %) and disturbance (30 %), and these effects did not depend on patch type (Table 3; Fig. 4). There was no significant interaction between sowing and disturbance. Regardless of treatment, *Lolium* patches remained significantly more species-rich than *Cynodon* and *Sorghum* patches (Tukey's test $P < 0.05$), while *Festuca* patches contained the lowest total richness (Table 3; Fig. 4).

Sowing did not significantly modify total live aboveground biomass (Table 3). Total live biomass was influenced by the varying impact of plant removals on exotic-dominated patch types (Table 3; Fig. 5). Disturbance did not affect community biomass in *Lolium* patches, whereas it strongly reduced final biomass of *Festuca* patches. In contrast, disturbance significantly increased total biomass in both *Cynodon* and *Sorghum* patches (Tukey's tests, $P < 0.05$; Fig. 5). Similarly, biomass removal decreased standing dead biomass by 40 % in *Festuca*, by nearly 25 % in *Sorghum* and *Cynodon*, and by less than 5 % in *Lolium* patches (patch × removal: $F_{2,36} = 3.0$, $P < 0.05$; data not shown).

Fig. 2 Effect of native grass seed addition and pulsed plant removals on species richness of sown native grasses (**a, b**), unsown native grasses (**c, d**), exotic grasses (**e, f**) and all forbs (**g, h**) in four exotic-dominated patch types. Values are means \pm 1 SE ($n = 4$) after 2 years of experiment. Patch types: *Lolium* (LOL), *Cynodon* (CYN), *Shorghum* (SOR), *Festuca* (FST)

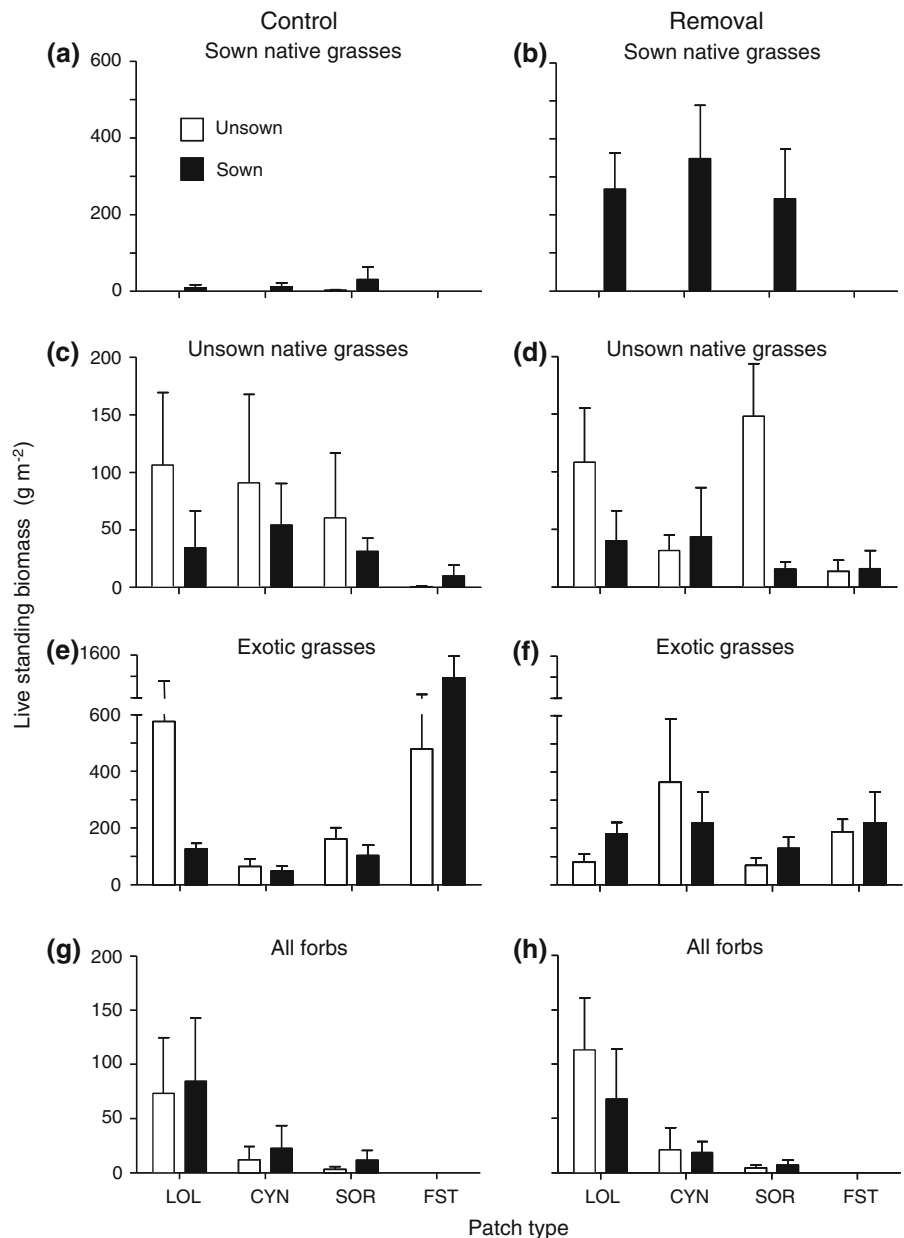


Discussion

Our results show that the establishment of native perennial grasses was co-limited by propagule arrival and site pre-emption by resident exotic species. Moreover, we found that once recruitment inhibition was relaxed by seed addition and a single-pulse disturbance, native grass performance depended on patch type, as defined by the local dominance of

different exotic grasses. This created a patchwork of native grass colonisation, with different sown species establishing in patches dominated by exotics of differing competitive ability (Table 2). Our study provides new evidence that invasive exotic species play a key role in shaping the structure and composition of 'novel' plant assemblages developing in modern anthropogenic landscapes (Hobbs et al. 2006; Cramer et al. 2008; Baer et al. 2009; Simberloff 2010).

Fig. 3 Effect of native grass seed addition and pulsed plant removals on live standing biomass of sown native grasses (**a, b**), unsown native grasses (**c, d**), exotic grasses (**e, f**) and all forbs (**g, h**) in four exotic-dominated patch types. Values show means \pm 1 SE ($n = 4$) after 2 years of experiment. Acronyms for patch types as in Fig. 2



Hierarchical limitation of native grass establishment

The assembly of new species into a local community is hierarchically constrained by dispersal from the regional species pool and niche limitations created by habitat conditions and biotic interactions (Ricklefs and Schluter 1993; Zobel et al. 2000; Foster et al. 2004; Myers and Harms 2009). This

framework is also appropriate to understand the barriers to native species re-establishment in heavily invaded systems (Cramer et al. 2008; Seabloom 2011). Here we found support to the hypothesis that resident exotic plants interact in a hierarchical fashion with seed availability in limiting colonisation of post-agricultural grasslands by native Pampa grasses. This interaction may explain the paucity of native grass species in long abandoned successional

Fig. 4 Effect of native grass addition and pulsed plant removals on plant species richness in four exotic-dominated patch types. Values show means +1 SE (n = 4) after 2 years of experiment. Acronyms for patch types as in Fig. 2

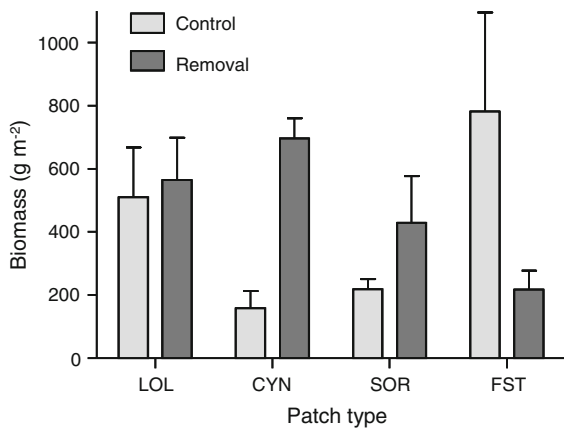
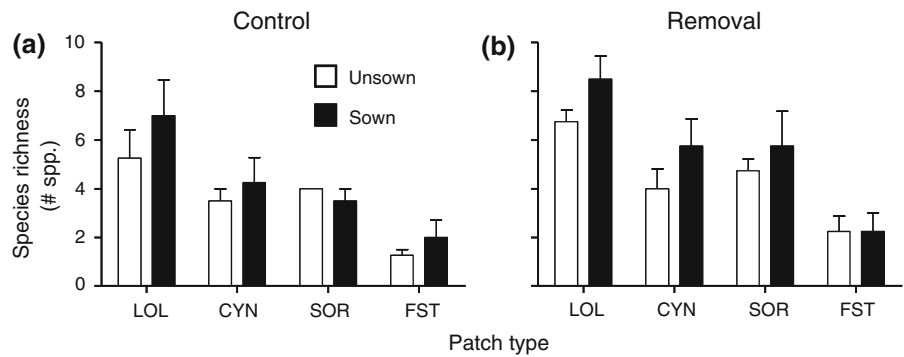


Fig. 5 Effect of pulsed plant removals on live standing biomass of four exotic-dominated patch types. Values show means +1 SE (n = 4) after 2 years of experiment, pooling over seed addition treatments. Acronyms for patch types as in Fig. 2

fields in the study region (Facelli et al. 1988; Omacini et al. 1995; Tognetti et al. 2010).

Seed addition was a necessary but not sufficient condition for the successful establishment of a pool of native grasses (Table 2; Figs. 1, 2, 3). Only the large-seeded *M. brasiliensis* recruited in sown/intact vegetation patches, albeit with low abundance. This implied that, regardless of dominant exotic species, all patch types were roughly equivalent at inhibiting native grass recruitment from added seed (Connell and Slatyer 1977). Further, plant removals alone did not promote colonisation of native perennial grasses, consistent with the idea that natives were strongly seed-limited in this old-field community (Eriksson and Ehrlén 1992; Turnbull et al. 2000). The lack of native grass seeds would result from either low arrival from distant sources or their absence in the soil seed bank (D'Angela et al. 1988), as well as reduced seed production from founder populations (Omacini et al.

1995; Münzbergová and Herben 2005). Other studies have shown the importance of dispersal limitation for native plant recovery in fragmented landscapes (Seabloom et al. 2003; Soons et al. 2004; MacDougall and Turkington 2005; Standish et al. 2007). Native tussock grasses common to pristine Pampa grasslands may nowadays persist in landscape refugia, including non-cropped lowland areas, old pastures, and narrow roadside and fencerow corridors (Poggio et al. 2010; Burkart et al. 2011).

Plant removals significantly enhanced native grass establishment after propagule limitation was reduced by seed sowing, a pattern common to other systems (Foster et al. 2004; Clark et al. 2007; Myers and Harms 2009). Although removals were applied as a single-pulse disturbance at the onset of the experiment, they effectively increased microsite availability creating a 'window of opportunity' for native grass recruitment. Both lack of seed germination beneath the litter layer and suppression of emerging seedlings by asymmetric competition from resident plants may have prevented recruitment of sown grasses (Tilman 1993; Foster and Gross 1997; Crawley et al. 1999). Consistent with the inhibition mechanism of succession (Connell and Slatyer 1977), recovery to native grass dominated communities may require frequent, small-scale disturbances to displace established exotic grasses. Hence, without human intervention, spontaneous colonisation of pampean old fields by native grasses would be unlikely (see also Seabloom 2011).

Interestingly, the fact that sown species successfully established in disturbed plots indicates that despite decades of agriculture local habitat conditions were still adequate for native perennial grasses. Thus, abiotic legacies from prior site management (e.g. altered soil properties) were not evident in our study (Cramer et al. 2008). We realise, however, that exotics

may still displace native grasses under present environmental conditions in the long run (see Daehler 2003). Old-field succession may be arrested in an exotic community state (Tognetti et al. 2010), as a consequence of biotic legacies created by habitat fragmentation and exotic species invasions (Seabloom et al. 2003; Cramer et al. 2008). Invasive exotic grasses appear to have a clear successional advantage over native perennial grasses, presumably due to their higher propagule pressure and rapid population growth. In our system, dominant exotic grasses are common weeds of winter (*Lolium*) and summer (*Cynodon*, *Sorghum*) crops, and are also widely sown as forage pastures (*Lolium*, *Festuca*). They are readily available to colonise fallow sites from nearby crop fields and pastures, as well as from remnant vegetative propagules. Long-term surveys in this system suggest that these exotic grasses may occupy different successional niches in the absence of indigenous grasses (Tognetti et al. 2010).

Identity effects in exotic-dominated patches

We found substantial variation in establishment of sown grass species across patches dominated by different exotics (Table 2; Figs. 1, 2, 3). For instance, sown species richness in removal plots decreased with the competitiveness of the exotic dominant (Fig. 2b), and was inversely related to patch differences in light interception. Yet, that patch identity effects were only apparent in removal plots suggests that microsite limitations to recruitment were revealed during post-disturbance dynamics (Symstad et al. 1998). Mechanisms underlying patch identity effects may be competitive, non-competitive (e.g. seed predation, see Maron and Gardner 2000; Orrock et al. 2009) or a combination of both (Hierro and Callaway 2003; Seabloom et al. 2003; MacDougall and Turkington 2005). If just competitive ability of exotic dominants had driven the observed patterns, we would expect to see differential recruitment of native grasses among intact patches (Tilman 1993; Foster et al. 2004). Contrarily, after 2 years, establishment of sown grasses within intact vegetation was similarly low in all patch types. This indicates that exotic residents were all competitive enough to inhibit native recruitment. It further suggests that factors other than competition affected microsite availability in

disturbed patches. We posit that functional differences in life history and phenology among exotic grasses might help to understand patch identity effects (Fargione et al. 2003; Wilsey et al. 2011).

In the absence of seed limitation, the addition of a native species to an exotic-dominated community must conform to the general invasion criterion (Chesson 2000). The introduced species must be able to increase from low abundance in the presence of other, resident plants. Coexistence of native and exotic grasses might be achieved through niche (e.g. phenological) differences that reduce potential overlaps in resource utilisation (i.e., a *stabilising* mechanism; Chesson 2000), or by factors that prevent competitive exclusion (e.g. disturbances) through a reduction of interspecific differences in fitness (an *equalising* mechanism). It thus seems inevitable that patterns of native recruitment were dependent on recipient patch identity, and were also largely individualistic (Table 2; see also Seabloom 2011). Seed addition and removals took place in late autumn, when cool-season exotics (*Lolium* and *Festuca*) were already growing actively, whereas warm-season species (*Cynodon* and *Sorghum*) were dormant. In this context, removal of warm-season perennial grasses provided the opportunity for invasion of several native winter (C_3) grasses, while disturbance of *Lolium* patches facilitated invasion by *P. quadrifarium*, an actively-growing native warm-season (C_4) grass.

On the other hand, *Festuca* dominated patches were not invasible at all, although our pulsed removals effectively decreased standing biomass for 2 years (Fig. 5). We expected cool-season exotic grasses to prevent recruitment of native C_3 grasses because of the large seasonal overlap between both species groups (Chesson 2000). However, when disturbed, annual *Lolium* patches were colonised by most added species, perhaps because the timing of removal determined that *Lolium* could only re-establish from seed the next year (Table 2; Figs. 2, 3). In contrast, rapid regrowth from perennial grass structures in *Festuca* patches may have drastically reduced the chances of successful colonisation by native grasses after disturbance. In this view, recruitment of native grasses arriving in exotic-dominated patches would reflect the interaction of stabilising and equalising mechanisms (Chesson 2000), as related to the species' phenologies and life-history traits (Wilsey et al. 2011, Wolkovich and Cleland 2011).

Community effects of native grass re-establishment

We found that, on average, adding native grass seeds increased total plant richness in both intact (26 %) and disturbed (23 %) patches. This effect did not extend to *Festuca* patches, although the sowing by patch type interaction was not significant (Tables 2, 3; Fig. 4). The introduction of dispersal-limited native grasses led to the reduction of other, established native grasses, which colonised naturally the study community (Table 3; Fig. 3). The paucity of dispersal of native perennial grasses into abandoned fields represents a serious concern given the extensive destruction of natural grasslands by human activity (Baldi et al. 2006). Our results imply that preserving refuge habitats as sources of native grass propagules is critical (yet not sufficient) to increase the chances of maintaining native plant biodiversity in the study region.

Unlike other grassland studies, seed addition did not enhance total community biomass in our study system (Table 3; Fig. 5). For instance, Foster et al. (2007) found a 300 % increase in grassland primary production with the addition of 32 species, while Stein et al. (2008) observed a 15 % biomass increase with the addition of 60 seed species. In these examples, added species managed to enter relatively intact plant communities. By contrast, Wilsey and Polley (2003) reported a reduction in grazed grassland productivity with seed addition, suggesting that added forbs interfered with resident *C₄* grass tillering. In our study, sowing only increased native biomass production after disturbance. Overall community changes after disturbance were only moderate (Fig. 1), as the early reduction of exotic dominants was compensated by other resident exotic grasses, as well as by sown grasses (Table 2). These results support the notion that niche overlap strongly affects coexistence in these novel assemblages, thus reducing the potential for complementary resource use among native and exotic grasses (Wilsey et al. 2009). Nevertheless, it is also possible that the addition of a small number of native grasses represented too low a functional diversity in the immigrant species pool (most were *C₃* grasses). There might not be a rigid upper ceiling to biomass production in native/exotic plant mixtures. Rather, the species we introduced could lack the appropriate traits to increase productivity through either niche

complementarity or selection effects (Loreau et al. 2001; Foster et al. 2004).

In conclusion, we have shown that re-establishment of native perennial grasses in an old-field grassland was constrained by exotic resident plants as well as by seed arrival. The observed between-patch variation in native grass recruitment suggests that communities dominated by exotics of differing competitive status (see Tognetti et al. 2010) create different opportunities for restoring native grass assemblages (Cramer et al. 2008; Baer et al. 2009). Invasive exotic plants may have entered the regional species pool as ‘passengers’ of anthropogenic environmental change (Didham et al. 2005). Yet, as implied by our findings and those of others (MacDougall and Turkington 2005; Vilá et al. 2011), exotics can turn into drivers of community dynamics by preventing the natural recovery of native species from remnant seed sources.

Acknowledgments We thank the staff at Estancia San Claudio for logistic assistance throughout this project. Two anonymous reviewers provided helpful comments to improve the manuscript. The study was funded by the University of Buenos Aires (grants no. G-024 and G-046). PMT was supported by a doctoral fellowship from Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) Argentina.

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