Community disassembly and invasion of remnant native grasslands under fluctuating resource supply

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Summary

1. Native vegetation fragments embedded in anthropogenic landscapes are increasingly threatened by land-use intensification. Managing disturbance regimes and nutrient inputs may help maintain species diversity in such remnants. Yet, it is unclear the extent to which changes in resource availability due to reduced capture by resident plants and/or increased supply rates may trigger native community disassembly and exotic invasions.

2. We examined how mowing disturbance and N fertilizer addition affected plant community recovery after a burning event in a remnant corridor of tussock pampa grassland in Argentina. The percentage cover and richness of native and exotic plant functional groups were monitored over 4 years. According to the 'fluctuating resource theory', we expected invasion to be the highest when both light and N availability were increased simultaneously.

3. Mowing delayed recovery by dominant C_4 tussock grasses and promoted subordinate, native C_3 grasses and exotic legumes, thus enhancing both native and exotic species richness. Fertilization induced a transient increase in native forbs but decreased total plant richness. Moreover, N addition to mowed grassland led to rapid invasion by short-lived exotic forbs, which were then replaced by exotic perennial grasses. Exotic grasses eventually spread across the grassland corridor, although at different rates depending on the treatment, and in parallel to a generalized decline in native species cover.

4. *Synthesis and applications.* Community disassembly patterns reflected differential responses of native and exotic functional groups to altered resource supply rates. Synergisms between canopy disturbances and N enrichment posed the greatest threat to preserving a pampa grassland remnant prone to invasion. Establishing buffer zones may be required to enhance the viability of corridor-like grassland remnants in agricultural landscapes.

Key-words: burning, disturbance, fertilization, functional groups, invasion, mowing, species richness

Introduction

Human-dominated landscapes may still contain remnants of native vegetation of high conservation value (Donald & Evans 2006; Ellis *et al*. 2010). However, natural habitat fragments are increasingly threatened by anthropogenic activities, including conversion to agriculture and domestic grazing (Ellis *et al*. 2010), land-use changes in the surrounding matrix (Leach & Givnish 1996; Wiens 2009) and invasion by exotic species (Cully, Cully & Hiebert 2003; Donald & Evans 2006; Didham *et al*. 2007). In particular, managing disturbance regimes and resource inputs in such vegetation relicts may be crucial for preserving their bio-diversity in the face of exotic invasions (Collins *et al*. 1998; Huston 2004; Clark & Tilman 2008). There is therefore a need to forecast how various anthropogenic stressors, acting solely or in combination, may weaken invasion resistance (Firn, House & Buckley 2010; Besaw *et al*. 2011) and trigger community 'disassembly' processes (Zavaleta *et al*. 2009) in native vegetation fragments embedded in agricultural landscapes.

Community disassembly is the non-random process of species decline and loss induced by a specific environmental driver (Zavaleta *et al*. 2009). During community disassembly, the local interaction network is reshuffled (Belyea & Lancaster 1999; Zavaleta *et al*. 2009), as former dominant species decline and the remaining subordinate species take advantage of newly available resources (Grime 1998).

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Disassembly trajectories can involve invasion by exotic species (Ellis, Antill & Kreft 2012), which may also act as drivers of habitat degradation for native species (MacDougall & Turkington 2005; Didham *et al.* 2007). It has been suggested that predictable trends in community composition or aggregate attributes (e.g. diversity) could arise depending on the factor, or set of factors, driving the disassembly process (Zavaleta *et al.* 2009). Such community patterns may be depicted by shifts in abundance of native and exotic functional groups, reflecting differential species' responses to major environmental gradients (Thompson *et al.* 2001; Chaneton *et al.* 2002; Huston 2004; Besaw *et al.* 2011). For instance, short-lived ruderal forbs are expected to thrive under disturbed, resource-rich conditions (Grime 2001), whereas legumes would be favoured in N-poor soils (Ritchie & Tilman 1995). By contrast, fast-growing grasses often become invasive in fertile soils with low to moderate disturbance (Huston 1994; MacDougall & Turkington 2005). Yet, whether divergent responses to disturbance and resources seen among functional groups overlap with species origin (native vs. exotic) is not entirely clear (Firn *et al.* 2011; Longo *et al.* 2013).

It is widely recognized that disturbance and resource supply play central roles in community structuring (Huston 1994; Grime 2001) and exotic invasions (Davis, Grime & Thompson 2000; Melbourne *et al.* 2007). The 'fluctuating resource theory' states that susceptibility to invasion increases with resource availability, or the amount of unused resources in a habitat (Davis, Grime & Thompson 2000). Invasibility often increases following disturbances that reduce resource capture by resident plants (Gross, Mittelbach & Reynolds 2005). Invasion may be also promoted by increased resource supply (Burke & Grime 1996; Daehler 2003), provided there is sufficient delay in the uptake of extra resources by resident plants (Shea & Chesson 2002). Moreover, the magnitude of invasion should be highest under the combined effects of disturbances and resource pulses (Davis, Grime & Thompson 2000; Thompson *et al.* 2001). This implies that community invasibility is not static but fluctuates over time (Davis & Pelsor 2001; Melbourne *et al.* 2007), a condition that should be common to grassland remnants (Leach & Givnish 1996; Wiens 2009). Thus, while disturbance can be essential to maintain diversity in native communities (Collins *et al.* 1998; Huston 2004), abrupt changes in resource availabilities may trigger disassembly trajectories by creating novel opportunities for exotic species (Shea & Chesson 2002).

The South American Pampas are one of the critically endangered grassland ecoregions of the world (Hoekstra *et al.* 2005). The region has been extensively transformed to agriculture, livestock grazing and urban or rural settlements (Baldi, Guerschman & Paruelo 2006). At present, only 5% of the original grassland biome is thought to have conservation value, and even <1% is pro-

tected (Bilenca & Miñarro 2004). In some areas, like the flat Inland Pampas, remnants of native grassland are extremely scarce, being confined to small fragments and corridors along fencelines, dirt roads and abandoned rail tracks (Bilenca & Miñarro 2004; Burkart *et al.* 2011). These remnants are often dominated by tall-tussock grass communities ('pajonales') that once covered extensive areas of the region (Perelman, Burkart & León 2003; Herrera *et al.* 2009). However, they are prone to invasion by exotic species when disturbed (Ortega & Laterra 2003; Burkart *et al.* 2011). Tussock grassland fragments are sporadically burned and grazed by cattle (Laterra *et al.* 2003), with still unknown consequences for their long-term viability as native preserves. Post-burn management may be crucial to invasion dynamics (Laterra 1997; Ortega & Laterra 2003), through its impact on dominant bunchgrasses and the supply rates of limiting resources (Mazía *et al.* 2010), including light penetration and soil nutrients (Davis, Grime & Thompson 2000; Gross, Mittelbach & Reynolds 2005; Besaw *et al.* 2011).

Here, we focus on two major issues relevant to protecting grassland remnants within a larger agricultural matrix (Ellis, Antill & Kreft 2012). Firstly, we asked how canopy disturbances and nutrient inputs interacted in changing the abundance of native resident species and exotic invaders after a burning event. Secondly, we examined which functional groups of native and exotic plants thrived under contrasting resource conditions. In a 4-year experiment, we tested the effects of mowing and N addition on post-burn community dynamics in a tussock grassland corridor, in the Inland Pampa, Argentina. We expected (i) annual mowing to increase light availability and slow the post-burn recovery of dominant tussock grasses while promoting subordinate, native or exotic species (Grime 1998; Longo *et al.* 2013); (ii) N addition to favour colonization by non-leguminous forbs and fast-growing (mainly exotic) grasses, which are common in neighbouring fields (Tognetti *et al.* 2010; Burkart *et al.* 2011) and (iii) the combination of mowing and fertilization to cause widespread invasion by various exotic functional groups, as predicted by theories emphasizing the role of fluctuating resources in plant invasion (Davis, Grime & Thompson 2000; Shea & Chesson 2002).

Materials and methods

STUDY AREA

The study was conducted in a remnant grassland corridor extending along the Midland Railway line (established 1910, discontinued in the 1970s) between the villages of Hortensia and Ordoqui, Carlos Casares county, Buenos Aires province, Argentina (35°53′ S, 61°05′ W). Mean annual precipitation is 1022 mm; mean monthly temperatures range from 23·4 °C in January to 8·2 °C in July. Dominant soils are well-drained, sandy-loam, Typic Hapludolls with *c.* 3% organic matter. The landscape is a mosaic of crop fields (soya bean, maize and wheat) under no-till agriculture, interspersed with sown pastures and semi-natural

grasslands managed for livestock grazing (Baldi, Guerschman & Paruelo 2006). The experimental site was a 400-m-long by 40-m-wide strip of tussock grassland bordered by a secondary dirt road and privately owned farmland. The site had not been grazed or burned for at least 20 years. Over the last 15 years, the adjacent fields were progressively converted from rangeland into cropland. Major threats to preserve such grassland corridors are created by agricultural intensification in the surrounding matrix and include occasional grazing, herbicide drift and nutrient run-off from fertilization. Indeed, sections of this grassland corridor have been burned and grazed or ploughed for field crops in recent years. Further, given its large edge-to-area ratio, it is conceivable that this grassland remnant receives abundant seed rain of weedy species from neighbouring fields (Mazía *et al.* 2010).

The plant community in the experimental site was dominated by the native C_4 bunchgrass *Paspalum quadrifarium* Lam., which accounted for 90% of the total above-ground biomass (*c.* 1700 g m^{-2}). The undisturbed grassland produced litter up to 800 g m^{-2} (Chaneton *et al.* 2004). Subordinate species comprised native perennial C_3 grasses (*Stipa* spp., *Piptochaetium* spp., *Melica brasiliana*, *Briza subaristata*), C_4 grasses (*Schizachyrium spicatum*, *Bothriochlora laguroides*) and several forbs (e.g. *Conyza bonariensis*, *Eryngium* spp.). Exotics included the perennial grasses *Festuca arundinacea*, *Sorghum halepense* and *Cynodon dactylon*, and the annual or biennial forbs *Carduus acanthoides*, *Cirsium vulgare* and *Conium maculatum* (Chaneton *et al.* 2004; Mazía *et al.* 2010). All these exotics are frequent components of old-field communities in the surrounding landscape (Tognetti *et al.* 2010).

The site was accidentally burned in spring (October) 2003. The fire eliminated most dead plant matter, creating large bare-soil spaces between the living remains of grass tussocks. The grassland had been surveyed for plant species composition and soil properties before the fire in December 2002 and was then resampled after the fire using the same protocol in January 2004 (see Appendix S1, Supporting information). These data provided a baseline to examine treatment effects on community structure and soil resources in the context of post-burn succession (see Appendix S1, Supporting information, for pre-treatment conditions).

EXPERIMENTAL DESIGN

In February 2004, 4 months after the fire, we established seven experimental blocks, irregularly spaced at 10–20 m along the grassland corridor. By using blocks at this scale, we intended to control for variation in topographic relief and dominant species cover. Each block comprised four 3 × 3 m plots, placed 3 m apart. Plots within blocks were randomly assigned to four treatments in a 2 × 2 factorial design (*n* = 7), with mowing disturbance (two levels, with/without) and N fertilizer addition (2 levels, with/without) as main factors. Mowing was applied once a year in April (autumn) and consisted of cutting all standing biomass to 5 cm height using a garden trimmer. Clipped biomass was raked off, and the litter was removed with minimum soil disturbance. Our mowing treatment was aimed to increase light penetration to the ground, thus mimicking the canopy disturbance created by domestic herbivores in tussock grassland relicts (Laterra 1997). Nitrogen was added as granulated ammonium nitrate at 45 g N m^{-2} year^{-1} and was applied in three equal doses in August (winter), December (spring) and March (summer) during 2004–2006. This protocol augmented N supply rates, simulating

anthropogenic inputs from cattle waste, fertilizer run-off and atmospheric deposition (Piñeiro, Paruelo & Oesterheld 2006). Overall, treatments were designed to alter resource availability through periodic reductions in the light intercepted by established plants and by pulsed addition of a limiting soil nutrient (Davis, Grime & Thompson 2000; Thompson *et al.* 2001).

DATA COLLECTION

We examined the effects of mowing and N addition on post-burn community dynamics during 2004–2007. The first sampling took place in May 2004, 3 months after the beginning of treatments. We sampled again in November (spring) and March (late summer) of each study year. Plant species cover was measured in two 1-m^2 quadrats, placed at the centre of each half of the plots. Percentage cover was visually estimated to the nearest 5% using a modified Daubenmire method (Tognetti *et al.* 2010). Data from the two quadrats were averaged within plots and then between dates within a study season (e.g. November 2004 and March 2005) to emphasize interannual changes in community structure rather than seasonal differences. We first looked into 'aggregate' community components including native species cover, exotic species cover and total species richness. We then evaluated the dynamics of various native and exotic functional groups. Species were classified into grasses (=graminoids), legumes and non-legume forbs and were labelled as native or exotic based on local floras (Zuloaga, Morrone & Belgrano 2008). For each functional group, we calculated the percentage cover and species richness (number) per plot and sampling date.

Above- and below-ground resource levels were monitored in six of the blocks on the same sampling dates as for the vegetation (May/2004, November/2004, March/2005, November/2005, March/2006, November/2006, March/2007). Light penetration (%) was measured at noon (11·00–13·00) during cloudless days using a 1-m-long ceptometer (Cavadevices, Buenos Aires, Argentina). For each plot, photosynthetic active radiation (PAR, μmol m^{-2} s^{-1}) was recorded once above the canopy and twice at ground level. Soil mineral N was measured for two 5-cm-diameter ×10-cm-deep soil cores taken from each plot. Mineral N fractions were determined from KCl 2 N extracts in an Alpkem analyzer (Wilsonville, OR, USA). Soil gravimetric water content (%) was assessed for two subsamples after drying at 105 °C. Plot subsamples were averaged before analysis. Climatic conditions during the experiment were relatively dry, as total rainfall for 2004 and 2005 was lower than the long-term mean (32% and 19%, respectively), while the fourth study year (2006–2007) was nearly average (1038 mm).

STATISTICAL ANALYSES

Plant cover and richness data were analysed through repeated-measures ANOVAS. Firstly, we performed a repeated-measures multivariate analysis (MANOVA) to account for the interactive nature of plant response variables under a given grouping criterion. These analyses focussed on the total cover of native and exotic species (two response variables) and the cover and richness of various functional groups (five response variables). Models tested for the fixed effects of block (*n* = 7), mowing and fertilizer (two levels each), and sampling date as repeated measure (four levels: May 2004, 2004–2005, 2005–2006 and 2006–2007). Secondly, we conducted univariate repeated-measures ANOVAS to examine the

responses of individual plant groups. The latter analyses tested separately for treatment effects on all natives, all exotics, native grasses, native forbs, exotic grasses, exotic forbs and exotic legumes. Native legumes were too rare to warrant analysis. When informative, we tested for short-term (3-month) treatment effects using data from May 2004 and a three-way ANOVA (block, mowing and fertilizer). Cover data were arcsine-square-root-transformed to meet the assumptions of ANOVA.

The analysis of resource data considered all seven (six for PAR) sampling dates, allowing for the full extent of resource fluctuations during the experiment. Light penetration, soil mineral N and soil water content were analysed using repeated-measures ANOVA, with block ($n = 6$), mowing and fertilizer (two levels each) as fixed effects, and sampling date as the repeated measure (six or seven levels). We also tested for mowing and fertilizer effects on spatio-temporal variation in resource levels (rather than mean values), by comparing the pooled variance of each abiotic variable (light, N, water) using variance-ratio tests (Zar 2010, p. 151). Variance estimations resulted from pooling blocks, sampling dates and factor levels ($n = 72$ or 84). Data analyses were performed using the General Linear Models module of STATISTICA software (StatSoft, Inc. 2007).

Results

RESOURCE DYNAMICS

Mowing and N addition independently altered light and soil resource availability throughout the experiment (Fig. 1, see Table S1, Supporting information). Mowing significantly increased light availability, whereas fertilization reduced light penetration at ground level (Fig. 1a). Light penetration in mowed plots decreased during the summer due to canopy recovery (Fig. 1a), which increased the spatio-temporal variation in light availability (pooled variance $F_{72,72} = 6.35$, $P < 0.001$). Fertilizer addition buffered temporal fluctuations in light penetration (Fig. 1a), but its effect on the pooled variance was not significant ($F_{72,72} = 1.32$, $P > 0.11$).

Soil mineral N content increased after each fertilization event in both mowed and unmowed plots, although the effect appeared to fade away with time since burning (Fig. 1b, Table S1, Supporting information). Still, fertilizer addition significantly increased spatio-temporal variation in soil N availability ($F_{84,84} = 16.41$, $P < 0.001$). Mowing did not significantly affect the average soil mineral N content or its pooled spatio-temporal variation ($F_{84,84} = 1.16$, $P > 0.24$; Fig. 1b, Table S1, Supporting information). Both mowing and N addition slightly reduced the average soil water content through time (Fig. 1c, Table S1, Supporting information), but did not alter its pooled variance ($P > 0.10$).

OVERALL COMMUNITY DYNAMICS

Mowing and fertilizer addition interacted in affecting total native and exotic plant cover during post-burn succession (M × F: $P < 0.003$, MANOVA, Table S2, Supporting infor-
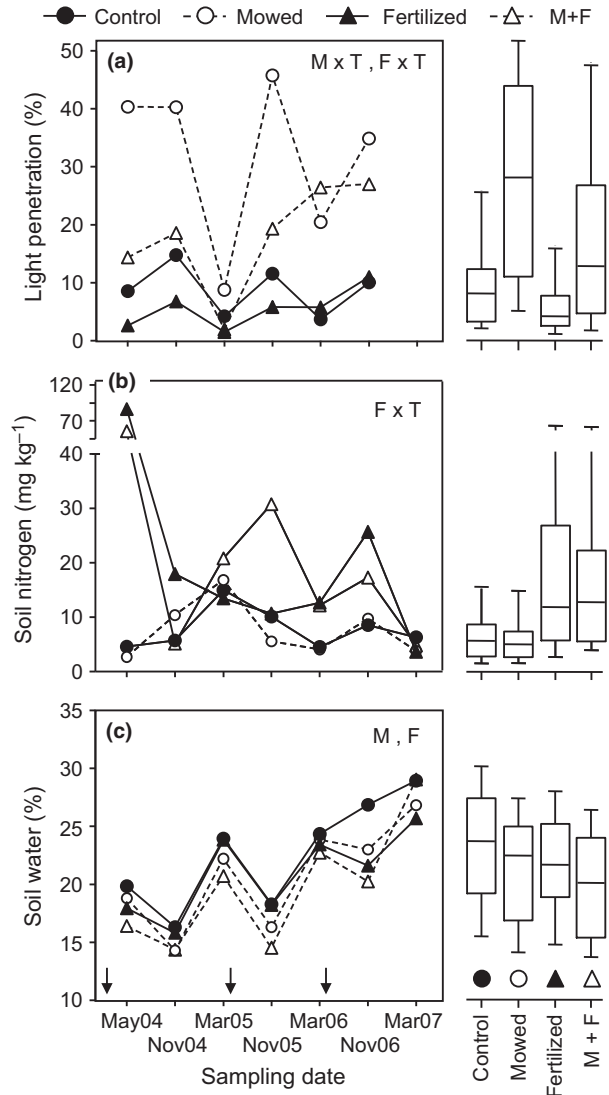

Fig. 1. Temporal variation in resource levels created by mowing and N addition during post-burn succession. (a) light penetration, (b) soil mineral N and (c) soil gravimetric moisture. Left panels: treatment means through time ($n = 7$). Right panels: box-and-whisker plots showing the overall variance of each treatment after pooling over blocks and sampling dates ($n = 42–48$). Light was not sampled in March 2007. Plots were left unmowed (solid symbols) or were mowed in autumn (open symbols); plots received fertilizer at 45 g N m^{-2} year^{-1} (triangles) or were not fertilized. Arrows indicate mowing events. Letters denote significant effects at $P < 0.05$ (M, mowing; F, fertilizer; T, sampling date).

mation). Moreover, treatment effects varied significantly through time (see Table S2, Supporting information), suggesting that native and exotic plants, overall, responded in opposite fashion to experimentally imposed changes in resource availability (Fig. 2). In the short term, mowing significantly reduced total native cover, regardless of fertilizer addition (May 2004, M: $F_{1,18} = 82.2$, $P < 0.001$, Fig. 2a), whereas mowing combined with fertilization caused a rapid increase in the extent of exotic plant invasion (May 2004, M × F: $F_{1,18} = 11.3$, $P < 0.001$,
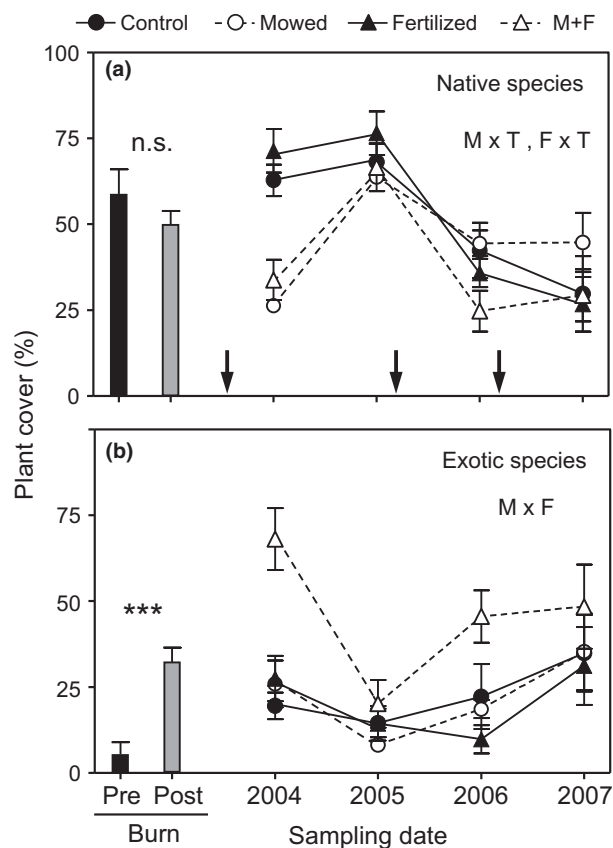
Fig. 2. Changes in plant cover in response to mowing and N addition during post-burn succession. (a) Total native plant cover, and (b) total exotic plant cover. Bars show pre- and post-burning means ($\pm$SE, n = 7), with significant differences (t-tests). Treatments were initiated in February 2004. Arrows indicate mowing events in late summer. Letters denote significant effects at P < 0·05 (M, mowing; F, fertilizer; T, sampling date). ***P < 0·001.
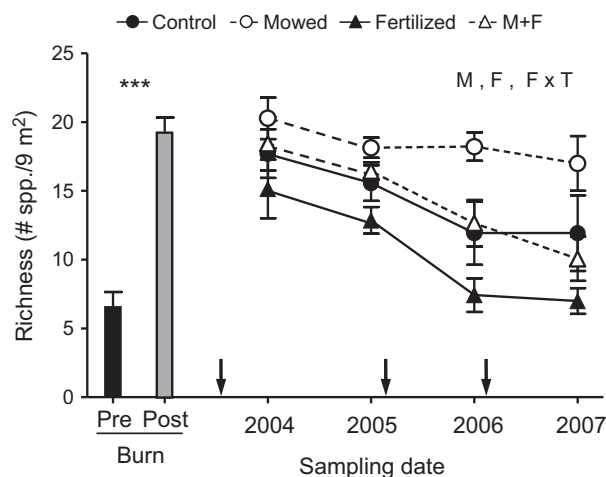

Fig. 3. Changes in total plant community richness in response to mowing and N addition during post-burn succession in a remnant of tussock grassland. Richness represents the number of species in 9 m² plots. Bars show pre- and post-burning means ($\pm$SE, n = 7), with significant differences (t-tests). Arrows indicate mowing events in late summer. Letters denote significant effects at P < 0·05 (M, mowing; F, fertilizer; T, sampling date). ***P < 0·001.

Fig. 2b). Native plant cover recovered from the initial mowing pulse, but then declined significantly across all treatments, and especially in the fertilized plots (Fig. 2a, Table S3, Supporting information). As a result, native species generally contributed <50% of the total community cover at the end of the study. Conversely, exotic plant cover tended to increase through time, with invasion being most apparent in mowed and fertilized plots (M × F: P < 0·002, Fig. 2b, Table S3, Supporting information).

In general, total species richness decreased with time since burning, yet species loss was more pronounced in fertilized grassland plots (Fig. 3, Table S3, Supporting information). This effect occurred as standing dead cover increased up to 50% in fertilized-only plots (repeated-measures ANOVA, $F_{1,18}$ = 9·4, P < 0·01). In contrast, mowing increased species richness relative to control plots throughout the experiment, regardless of fertilizer addition (Fig. 3). Mowing helped to maintain the elevated plant richness found after the fire in unfertilized plots (see Fig. 3).

FUNCTIONAL GROUP RESPONSES TO TREATMENTS

Multivariate analysis revealed highly significant effects of mowing and fertilization on the overall dynamics of functional group composition through 4 years of post-burn succession (both effects P < 0·001, MANOVA, Table S2, Supporting information). In addition, there was a strong mowing-by-fertilizer interaction (P < 0·01), indicating that plant functional groups responded differently and interactively to the combined influence of canopy disturbance and N addition, both in terms of ground cover and species richness (Fig. 4).

In the short term, mowing prevented the recovery of native grass cover to pre-burn levels (May 2004: $F_{1,18}$ = 60·6, P < 0·0001, Fig. 4a). In the following years, native grasses exhibited a general decline in cover (from *c*. 60% to 30%), irrespective of treatment (Fig. 4a, Table S4, Supporting information). This trend comprised a shift in the relative abundance of formerly dominant and subordinate native grass species. The dominant C₄ grass *P. quadrifarium* decreased across all treatments, but mostly in mowed grassland plots (M × T: P < 0·001; see Fig. S1, Supporting information). Conversely, mowing alone increased the cover of several native C₃ perennial grasses (M × T: P < 0·05), whereas N addition suppressed these species (F × T: P < 0·01, Fig. S1, Supporting information). Only the short-lived, C₃ native grass *Bromus catharticus* consistently increased within fertilized plots (see Fig. S1, Supporting information). On the other hand, for 2 years of post-burn succession, native forb cover remained higher in fertilized, relative to unfertilized plots (Fig. 4c). Yet, this pattern was reversed in the ensuing years as native forbs generally declined, except in mowed plots (Fig. 4c, Table S4, Supporting information).
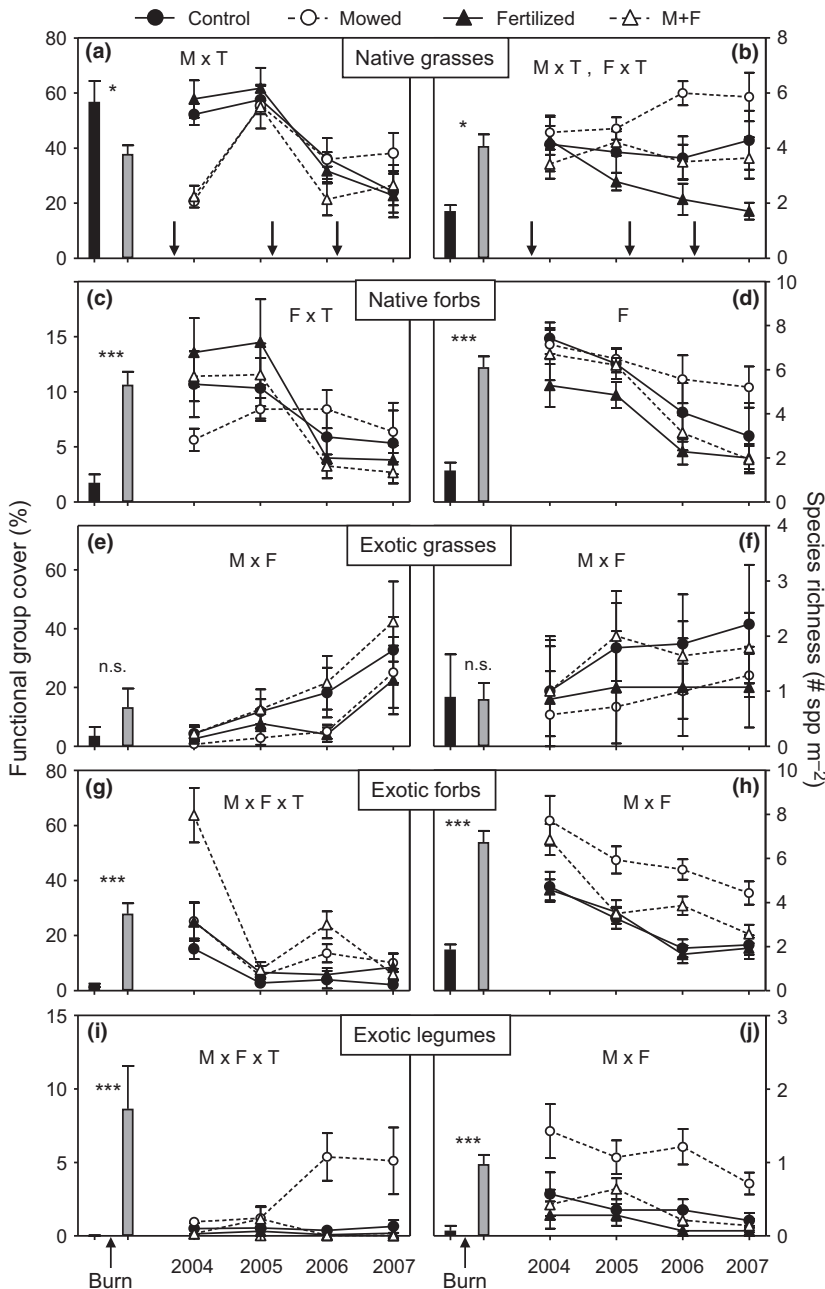
Fig. 4. Changes in plant cover (left panels) and species richness (right panels) for native and exotic functional groups in response to mowing and N addition during post-burn succession in remnant tussock grassland. (a,b) native grasses, (c,d) native forbs, (e,f) exotic grasses, (g,h) exotic forbs and (i,j) exotic legumes. Bars show pre- and post-burning means (±SE, $n = 7$), with significant differences (*t*-tests). Arrows indicate mowing events in late summer. Letters denote significant effects at $P < 0.05$ (M, mowing; F, fertilizer; T, sampling date). *$P < 0.05$, ***$P < 0.001$.

Mowing and fertilizer addition interacted synergistically to increase the cover of non-legume exotic forbs at different stages of post-burn succession (Fig. 4g, Table S4, Supporting information). In the short term, adding N to mowed plots promoted a threefold increase in exotic forb cover, relative to the control grassland (May 2004: M × F: $F_{1,18} = 6.8$, $P < 0.02$). This effect decreased over time but remained significant after 3 years (Fig. 4g). By contrast, exotic grasses and legumes did not respond to treatments in the short term (May 2004: *P* values > 0·10). In the longer term, however, mowing promoted invasion by exotic legumes in unfertilized plots, an effect that increased through time (Fig. 4i, Table S4, Supporting information). Intriguingly, exotic grass cover increased

across all treatments after 4 years of experiment (Time: $P < 0.001$). Exotic grass invasion was accelerated by the combined effects of mowing and N addition, while both factors slowed down invasion when acting alone (Fig. 4e, Table S4, Supporting information). *Festuca arundinacea* was the dominant exotic grass, reaching up 50% cover in some plots (see Fig. S1, Supporting information).

Overall, mowing increased whereas N addition decreased species richness within functional groups, except for exotic grasses (Fig. 4, Table S4, Supporting information). On average, mowing increased native grass richness by 4 and 2 spp m^{-2} in unfertilized and fertilized plots, respectively; fertilization reduced native grass richness by 2 spp m^{-2} (Fig. 4b). Native forb richness decreased with

time since burning, except in mowed-only plots, and declined more rapidly with N addition (Fig. 4d). Exotic grasses comprised only 1–3 spp m^{-2}; yet, adding N fertilizer significantly increased exotic grass richness in mowed plots and decreased it in unmowed plots (Fig. 4f). Lastly, mowing disturbance increased the local richness of exotic forbs and legumes, whereas fertilizer addition to mowed plots precluded this effect (Fig. 4h,j).

Discussion

Post-burn recovery of a corridor grassland remnant in the Inland Pampa was strongly affected by resource manipulations, in accordance with the fluctuating resource theory of plant invasion (Davis, Grime & Thompson 2000). We found that the loss of native grass dominance and widespread exotic species invasion were most pronounced when resource levels were altered through both decreased capture by established plants and increased supply from an external source (Burke & Grime 1996; Thompson *et al.* 2001; cf. Gross, Mittelbach & Reynolds 2005). Moreover, different combinations of disturbance and N subsidy triggered alternate disassembly trajectories (Lepš 2014), which varied in the relative roles of different exotic functional groups (Fig. 4). In our system, native and exotic functional groups appeared to respond differently to shifting resource conditions (Fig. 5), which could be a signature of other remnant grasslands (Smith & Knapp 1999; Cully,

Cully & Hiebert 2003; Besaw *et al.* 2011; Firn *et al.* 2011). We suggest that management decisions after major disturbance events can be critical to the long-term viability of grassland fragments in anthropogenic landscapes.

COMMUNITY DISASSEMBLY UNDER FLUCTUATING RESOURCES

Theory emphasizes that opportunities for exotic species establishment and spread are generated by the imbalance between gross resource supply and uptake by the resident plant community (Shea & Chesson 2002). The fluctuating resource theory posits that either intermittent or lasting increases in resource availability permit invasion and that combined reductions in resource capture and elevated supply should maximize the chances of invasion (Davis, Grime & Thompson 2000; Davis & Pelsor 2001). Our experimental results supported this view, since the largest extent of invasion, in terms of exotic species cover, occurred when N fertilizer was added to mowed grassland plots (Figs 2 and 4).

Mowing created marked spatio-temporal heterogeneities in light penetration (Fig. 1), which apparently provided suitable conditions for species that were otherwise suppressed by tall-tussock grasses (Smith & Knapp 1999). In addition, fertilization alleviated the low levels of soil available N found in the study grassland (Mazía *et al.* 2010), which would reflect efficient N uptake and retention by dominant C$_4$ grasses (see below). In the short therm, enhanced resource conditions were capitalized by short-lived, non-legume exotic forbs, whereas in the longer term, these colonizers were replaced by fast-growing exotic grasses like *F. arundinacea* (Grime 2001; Fynn, Morris & Edwards 2005). Applying higher disturbance rates by mowing (or grazing) would further relax above-ground competition, leading to alternative states dominated by low-growing forbs (Huston 1994; Chaneton *et al.* 2002; Fig. 5). We expect, however, that conditions increasing susceptibility to invasions should be system specific and may thus peak under disparate combinations of resource fluctuation (Davis & Pelsor 2001) or enrichment (Huston 2004; Firn, House & Buckley 2010).

Elevated N supply rates alone did not promote exotic species beyond the level observed in control plots (Figs 2 and 4). The dominant perennial grass *P. quadrifarium* and the short-lived native grass *B. catharticus* increased in cover within fertilized-only plots, yet at early and later stages of post-burn succession, respectively (Fig. S1a,e, Supporting information). Moreover, N fertilization increased the ground cover of dead plant matter, suggesting an acceleration of established grass growth and senescence. Rapid pre-emption of extra available N by native plants may preclude invasion until more N demanding exotic species eventually find an opportunity to become established (Shea & Chesson 2002). These results seem to contradict prior evidence for increased performance of exotic species in N-enriched environments (Daehler 2003)
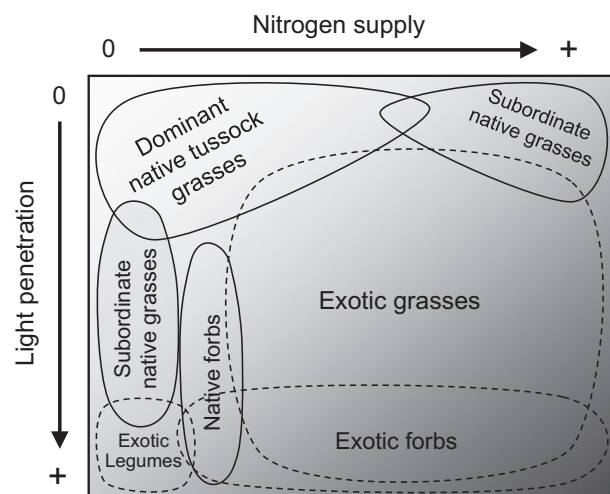

Fig. 5. Schematic summarizing the responses of native and exotic functional groups to changes in light and nitrogen availability in a tussock pampa grassland relict. The undisturbed system is dominated by native tussock grasses under low resource levels (top-left). Increasing light (bottom-left) or nitrogen (top-right) supply facilitates various subordinate native groups but also shifts the dominance towards different exotic groups. The magnitude of invasion increases along the diagonal (grey shading), as predicted by the 'fluctuating resource theory' (Davis, Grime & Thompson 2000). Notice, however, that the highest plant diversity is achieved under increased light penetration (canopy disturbances) and low N supply.

and point towards the potential role of resource colimitation in controlling invasion dynamics.

The reductions in resource capture by dominant C_4 grasses determined by annual mowing initially led to divergent patterns of community disassembly, involving the expansion of subordinate, native perennial grasses and forbs (Fig. 4, Fig. S1, Supporting information). Similar results have been reported for other grasslands in which selective disturbances nearly caused the extirpation of dominant species (Grime 1998; Longo *et al.* 2013; Lepš 2014). Without extra N inputs, canopy disturbances did not affect total native cover (Fig. 2), but induced a shift in relative abundance of resident native grasses. Remnants of tussock pampa grasslands comprise several C_3 bunchgrasses ('flechillas'), which become dominant under light grazing regimes (Perelman, Burkart & León 2003; Herrera *et al.* 2009). Our study suggests that subordinate grasses are competitively suppressed and thus respond to resources released by the removal of *P. quadrifarium*. Similarly, native forbs showed a positive response to mowing, which suggests that they are relatively less limited by N availability than their exotic forb counterparts (Figs 4 and 5). The increased cover of native subordinate species under low N supply may limit the opportunities for exotic species establishment created by intermittent canopy disturbances (Grime 1998; Shea & Chesson 2002).

Nevertheless, mowing promoted exotic legumes that were locally rare but common in sown grazed pastures and early successional fields in the study area (Burkart *et al.* 2011). The increased abundance of N-fixing exotic forbs during post-burn succession had been reported for other pampa grasslands managed with prescribed burning (Laterra 1997; Ortega & Laterra 2003), as well as in herbaceous systems elsewhere (Ritchie & Tilman 1995; Carino & Daehler 2002). Legumes may have an advantage over potential invaders in soils with low N availability because of their capacity to fix atmospheric N. However, the high energetic costs associated with symbiotic N fixation imply that legumes should perform better under increased light levels (De Tezanos Pinto & Litchman 2010), such as those created by our mowing treatment.

MAINTAINING SPECIES RICHNESS IN GRASSLAND REMNANTS

Remnant fragments and corridor habitats are havens for native plant diversity in agricultural landscapes like the Inland Pampas (Burkart *et al.* 2011). Land-use intensification puts these remnants at risk through spillover effects from human activities in the surrounding matrix (Didham *et al.* 2007; Wiens 2009). Eutrophication from increased fertilizer use in adjacent fields as well as atmospheric deposition can represent major threats to biodiversity (Clark & Tilman 2008). In general, N addition increases herbaceous species biomass, leading to increased competition and greater recruitment limitation via litter accumulation (Grime 2001; Suding *et al.* 2005). Greater biomass production may increase local extinction rates through random community thinning or trait-based, selective species losses (Suding *et al.* 2005; Dickson & Gross 2013).

Here, we found that N addition reduced total plant richness by nearly five species per plot, regardless of the positive effect of mowing on several plant groups (Figs 3 and 4). In our case, fertilization effects on species richness appeared to be largely unselective (Suding *et al.* 2005), involving both native and exotic species in various functional groups. Recent work shows that fertilization may decrease species richness even when competition for light is not a limiting factor to coexistence (Dickson & Foster 2011). The fact that mowing did not counteract the negative impact of N addition suggests that other mechanisms, possibly competition for soil resources (e.g. water; Fig. 1c) may contribute to reduce plant richness in N-enriched grasslands (Dickson & Foster 2011; Dickson & Gross 2013). These findings stress that N subsidies may threaten grassland diversity beyond management practices aimed at balancing competition between dominant and rare species (Collins *et al.* 1998; Huston 2004).

Interestingly, increasing above- and below-ground resource availability affected native and exotic forb richness in different fashions. While native species responded additively to mowing and fertilization, exotics forbs showed a non-additive response (Fig. 4). Pampean species pools comprise more exotic than native annual/biennial species (Chaneton *et al.* 2002; Tognetti *et al.* 2010). The fact that most exotic species should re-establish annually suggests that they are more sensitive to biomass accumulation under high N supply and may depend on periodic disturbances for persistence (Longo *et al.* 2013; see Fig. 5). Native and exotic forbs also exhibited distinct patterns of species decline during old-field succession (Tognetti *et al.* 2010). Overall, results suggest that the dynamics of native and exotic forbs present in this system may be governed by different limiting factors.

MANAGING REMNANT GRASSLANDS FOR CONSERVATION

Recognizing whether exotic invaders drive community disassembly, or are just better suited to thrive in human-disturbed environments, is essential to protect native remnants (MacDougall & Turkington 2005; Didham *et al.* 2007). In our system, exotic forbs succeeded only in highly modified grassland plots, in accordance with the passengers model (Fig. 5). In contrast, exotic perennial grasses eventually increased over the whole experiment including intact plots (Fig. 4, Fig. S1, Supporting information). This suggests that exotic grasses could operate as drivers of grassland disassembly during post-burn succession. The increasing dominance of old-field communities by introduced pasture grasses (Tognetti *et al.* 2010) is consistent with the notion that exotic grasses might contribute to the observed loss of species diversity in remnant

pampa grasslands. We noticed, however, that exotic grasses invaded after a marked decline in native grass cover that followed two dry years (Fig. 4a,c). Hence, the overall shift in grass composition observed in this experiment may have also reflected the effect of unmanipulated external drivers on both native and exotic species performance (Didham *et al.* 2007).

Our results have implications for managing remnant grasslands within conservation schemes. Firstly, retaining a species-rich ensemble of subordinate grasses may ensure greater stability in resource capture, thus providing an 'invasion resistance service' (Grime 1998; Shea & Chesson 2002). This may be achieved through a light disturbance regime, which periodically opens the established canopy. For instance, grazing with low N additions was an effective strategy to improve native grasslands in Australia (Firn, House & Buckley 2010). Secondly, our findings stress the importance of avoiding severe canopy disturbances on nutrient-enriched sites, as these conditions would facilitate invasion. Thirdly, N was a key factor determining whether post-fire dynamics led to recovery of native grass dominance or the community was deflected towards an exotic-dominated state. Hence, preventing N subsidies or reducing soil N availability (via C addition; Cherwin, Seastedt & Suding 2009) after large disturbance events may be critical to protect remnant grasslands from widespread invasion. Lastly, there is an urgent need to increase the 'effective' area of these remnants, by creating buffer zones to help reducing edge effects from the surrounding agricultural matrix (Wiens 2009).

In conclusion, our study illustrates the fragile condition of these corridor-like grassland relicts, where burning may trigger a disassembly process, partly driven by exotic invasions and management decisions, both on and off the focal fragment. For remnant grasslands to persist as viable biodiversity reserves within agricultural landscapes, land managers should avoid creating novel resource opportunities for invasive exotic species.

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Data accessibility

Data archived in Dryad Digital Repository: DOI:10.5061/dryad.46181 (Tognetti & Chaneton 2015).

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Sampling methods and results describing effects of a burning event on remnant grassland vegetation and resource availability before the start of the experiment.

Table S1. ANOVA table showing the effects of mowing and fertilization on percentage light penetration, soil mineral nitrogen and soil water content.

Table S2. Multivariate ANOVA showing the overall effects of mowing and fertilization on plant community composition and species richness.

Table S3. ANOVA table showing the effects of mowing and fertilization on native and exotic species cover and total plant species richness.

Table S4. ANOVA table showing the effects of mowing and fertilization on the cover and richness of native and exotic functional groups (grasses, forbs and legumes).

Fig. S1. Changes in the cover of major native and exotic grass species in response to mowing and fertilization during post-burn succession.