Local and regional long-term diversity changes and biotic homogenization in two temperate grasslands

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Abstract

Questions: Are there losses in species richness due to extinctions? Is the input of new species lower in environments with strong resource limitation? Is there a decrease in abundance and diversity of native species due to anthropogenic disturbance at local and regional scale? Has abundance of exotic species been favoured by grazing increased? Are floristic changes associated with homogenization processes?

Location: Two grassland community types of the Argentine Flooding Pampas: the mesophyte prairie and the halophyte steppe.

Methods: Grassland sites ($n = 51$) were resurveyed with the same methodology after 35 yr. At the local scale (0.25 ha), we analysed alpha diversity, site colonization and extinction rates, and changes in species and functional type composition. Site colonization rates were calculated separately for new species (not recorded in the original inventory) and old species (present in the first survey at other sample sites of the community). Correspondence analysis was applied to the presence/absence species matrix for all sites in both periods. At regional scale, we evaluated gamma diversity, heterogeneity within and between communities, changes in proportion of functional types (growth season, longevity and life form) and species origin.

Results: In the mesophytic prairie, average local species richness increased 46% and regional richness 28%, while in the halophytic steppe changes were not significant. The colonization rate was higher and the extinction rate lower in the richest community (mesophytic prairie). Differences in colonization rates between natives and exotics were not found. Principal variability between sites in species composition was associated with abiotic conditions explaining 20.9% of inertia, temporal changes were relegated to the second axis with 6.38%. Proportions of warm-season exotics among new arrivals were higher than expected. Biotic homogenization was evidenced in the mesophytic prairie as a significant reduction in species turnover between sampling dates.

Conclusions: After 35 yr under moderate grazing intensity we found increases in species richness, no biodiversity loss and no losses of native species. Instead of those species favoured by grazing, as expected, warm season species increased. Different grassland communities showed clear differences in richness changes, in degree of homogenization and in species turnover.

Introduction

The successional trajectory of natural grasslands across the world has been altered by human activities such as grazing by domestic livestock or cultivation, which can promote anthropogenic species loss, exotic species invasion and landscape fragmentation. Such processes, together with other main drivers of global environmental change, e.g. CO_2 enrichment and associated climatic changes, are all predicted to become more important as human exploitation of the environment increases over short time scales (Sala et al. 2000; Pereira et al. 2010; Bellard et al. 2012). The importance of managing species invasions, extinctions and large changes in species composition in nearly every

major ecosystem should make community-level scaling a strong research priority in ecology (Suding et al. 2008; Mokany & Ferrier 2011). In this context, repeated bio-diversity surveys of different environments are expected to play an important role in understanding the effect of regional and local processes on biodiversity and ecosystem function losses at different scales (Schlapfer & Schmid 1999; Bai et al. 2004; Tilman et al. 2006).

The regional plant diversity of a community can be increased by species input from other regions or other communities within the region, and decreased by extinction. Local diversity may be highly dependent on regional diversity, which determines the size of the species pool available to colonize new sites (Stohlgren et al. 2008). If we consider community sites as partially isolated homogeneous environments, changes in local species richness can be viewed, in terms of island biogeography theory, as a balance between local colonization and extinction rates (MacArthur & Wilson 1967). Abiotic stress, local interactions and disturbance regime will also influence these rates, i.e. the extinction rate can be increased by environmental stress, competitive exclusion or intensive disturbance. The colonization rate can be enhanced by input of propagules of new species, availability of regeneration niches mediated by moderate disturbance, or facilitation by organisms that protect from herbivores, climate extremes and poor resource availability (Olff & Ritchie 1998; Brooker et al. 2008). As species losses and colonization occur simultaneously, they may determine any of both increases and decreases in community diversity at regional and local scales (Sax & Gaines 2003; Wardle et al. 2011).

Several studies have shown no changes over time in species richness at regional scale but significant changes at site scale (Stohlgren et al. 1999; Keeley et al. 2003). When the regional richness remains unchanged, local or alpha diversity may increase at the expense of a decrease in beta diversity (species turnover among sites), a process known as biological homogenization (Olden & Rooney 2006). This process, a common consequence of human activities, promotes local extinction of native species and their replacement by exotics that are widespread among communities (McKinney & Lockwood 1999; McKinney 2004). Despite compositional turnover, no changes in local and regional richness are expected if species saturation occurs (Elmendorf & Harrison 2011); but if saturation is not achieved, then regional and local species richness could increase (Sax & Gaines 2008).

The impact of biological invasions on community dynamics and ecosystem function is increasingly recognized as being correlated with the change induced by new species on functional group composition (Wardle et al. 2011). Increasing evidence suggests that plant functional group diversity and composition may be more important

to ecological integrity than plant species diversity, as it affect ecosystem stability and resource dynamics (Loreau 2000; Diaz & Cabido 2001; Pokorny et al. 2005). Functional traits related to life-history strategies, such as life longevity and growing season, determine the timing of species establishment and possibly competitive outcomes (Byun et al. 2013). It is therefore important to register changes in the proportion of functional groups based on relevant traits in order to understand community dynamics. Site resampling and comparisons are necessary tools to accurately detect those regional changes that determine plant species and functional group composition.

We examine the pattern of long-term vegetation change in two grassland community types of the Argentine Flooding Pampas (Soriano et al. 1992). Human intervention and grazing by domestic cattle over the last four centuries resulted in the massive invasion of these grasslands by exotic plant species, which today account for ca. 25% of the regional vascular flora (Hudson 1892; León et al. 1984; Perelman et al. 2001). Due to soil limitations and frequent flooding, less than 20% of the area has ever been cultivated, while most of it is used for cattle husbandry on non-fertilized natural grasslands (León et al. 1984; Soriano et al. 1992). In this region, grassland species composition varies across the landscape in relation to differences in topography and soil salinity (Perelman et al. 2001). Community types are associated with different combinations of these environmental variables, the more contrasting being the mesophyte prairie (MP) and the halophyte steppe (HS). As environmental variation is relatively fine-grained, stands of these and other community types occur within relatively small areas (<10 km^2) in a complex landscape mosaic repeated across the entire region (Fig. 1; Perelman et al. 2001). In these grassland types, abiotic constraints imposed by frequent flooding and elevated soil salinity
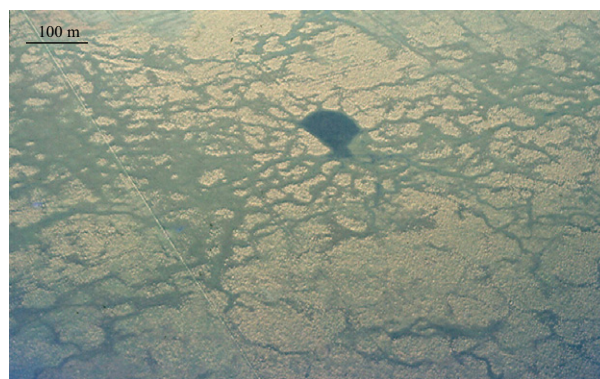

Fig. 1. Aerial view of a paddock showing the distribution pattern of the two community types: the mesophytic prairie (lighter) and the halophytic steppe (darker) in this area of the Flooding Pampa, Argentina. Photo R. J. C. León.

may limit community invasibility (Dethier & Hacker 2005; Von Holle 2005) and the persistence of both native and exotic species. The low tolerance of many plants to soil salinity should decrease the colonization rate and increase the extinction rate in halomorphic sites.

In these two communities we studied changes over the last decades in species richness and composition at local and regional scale. We expected losses in species richness due to local extinctions in both community types, and less input of new species in sites with strong resource limitation for their establishment and growth (Stohlgren et al. 2008). Despite the intensity of changes in total richness, we expected to find a decrease in abundance and diversity of native species due to anthropogenic disturbance (essentially grazing) both at local and regional scale, coupled with increased exotic species abundance, mainly from functional types favoured by grazing (herbs, annuals). In consequence, we envisaged species homogenization within and between communities as a consequence of an increase in abundance of generalist exotic species (Olden & Rooney 2006).

Methods

Study area

The study area comprises around 100 000 ha in the northern portion of the Flooding Pampa (35°17′ S, 57°30′ W). The climate is temperate-humid, with a mean annual rainfall of 1025 mm and an average annual temperature of 16.3 °C. The landscape is characterized by its extremely flat topography and treeless physiognomy. These grasslands are only interrupted by roads, railways, some agricultural plots, short streams and ponds (Baldi et al. 2006). Except on the higher portions of undulating landscapes with well-drained soils, drainage is poor due to lack of slope and reduced infiltration. The vegetation is arranged as a complex mosaic of herbaceous plant communities: mesophyte prairies, humid prairies and halophytic steppes (León et al. 1979). Species composition responds to local variations in topography and soil salinity, but has also been shaped by a century of livestock grazing (León et al. 1984; Sala et al. 1986). The plant communities we studied were the mesophyte prairie: grasslands on well-drained Argiudoll soils, and the HS: grasslands on saline/alkaline lowlands on Natraqualf soils. Mesophyte prairie patches are larger than those of the HS and show the highest annual productivity with the lowest interannual variability among all the plant communities of this area (Aragón & Oesterheld 2008). In contrast the halophytic steppe is the less productive community. Grazing intensity by domestic cattle is similar among the different grassland communities, with a stocking rate of around 0.55 cow ha^{-1}·yr^{-1}.

Field data

A phytosociological survey was conducted in 1968 (León et al. 1979) in different grassland habitat types that had been under continuous cattle grazing. Vegetation samples were located to represent the previously identified physiographic units, excluding situations with current or recent agricultural use (<5–10 yr). In 2003, using precise maps to be able to locate them accurately, 51 of the 148 grassland sites sampled in 1968 were resurveyed using the same methodology. Twenty-nine of these belonged to the MP, the other 22 sites to the HS (Perelman et al. 2001). Each vegetation sample consisted of a complete list of the vascular plants present in the whole site (ca. 0.25 ha), and visually estimated percentage aerial cover of each individual species using a modified Braun-Blanquet abundance scale (Mueller-Dombois & Ellemberg 1974) within a 5 m × 5 m area, as in the previous inventory. Researchers that conducted the first survey in 1968 were part of the team that carried out the sampling of this second one. In this way we expected to decrease possible errors associated with differences in species recognition and sampling effort. On both occasions sampling was done in early summer, the moment when both cold and warm season species are easily recognizable. Plant species nomenclature follows Zuloaga & Morrone (1996/1999) and Zuloaga et al. (1994).

Data analysis

At local scale, the units of analysis were each vegetation site (0.25 ha). We analysed alpha diversity, site colonization and extinction rates and changes in species and functional type composition in those units. At regional scale, we considered all sample sites of each community type across the whole study region, and evaluated gamma diversity, heterogeneity within and between communities and changes in proportion of functional types over time. In order to quantify changes in community species number and composition, we used as starting reference all sites surveyed in 1968 (91 MP sites and 57 HS sites) as they provide a better description of the species present in the study region at the date of the first survey. This allowed us to identify among the species that arrived to the resurveyed sites: new species in the community (absent in 1968) and old species already present in the sites of the community that were not resurveyed in 2003.

Differences over time in mean species richness per site (α_i) in both communities were tested with the paired Student t-test. Change in species richness ($\Delta\alpha_i$) at each site i was quantified as the difference between the number of new species present in 2003 (A_i) and those that disappeared between the two sampling dates (E_i); extinction (e_i)

and colonization rates (a_i) are expressed in relative terms of total species available at each site (Eq. 1):

$$a_i = \frac{A_i}{\gamma_0 - \alpha_i} \quad e_i = \frac{E_i}{\alpha_i} \tag{1}$$

where γ_0 is the pooled species number across all sampled sites on both sampling dates and a_i is the number of species per site in 1968. Mean colonization (a_i) and extinction rates (e_i) were tested with the Wilcoxon test to evaluate rates of gain and loss at local scale between communities.

Site colonization rates were calculated separately for species already present in 1968 (a_{1i}) and new species (those that appeared in 2003) in the community (a_{2i}) (Eq. 2):

$$\Delta\alpha_i = a_{1i}(\gamma_1 - \alpha_i) + a_{2i}(\gamma_2) - e_i(\alpha_i) \tag{2}$$

where γ_1 is the pooled species number across all sites in 1968 and γ_2 is number of species only present in 2003.

In order to identify the pattern of variability in floristic composition of these grasslands, correspondence analysis (CA) was applied to the presence/absence species matrix for all sites in both periods (Legendre & Legendre 1998). Species were described according to: origin (exotic–native), growth season (warm vs cold), longevity (annuals vs perennials) and life form (herbs vs grasses). To determine if any of these groups of species was associated with changes in composition we compared the proportional contribution to richness by origin and each functional type, for both sampling dates, using the paired Wilcoxon test. To analyse changes in species origin and functional type composition as a whole, we applied multi-response permutation procedure (MRPP; McCune & Grace 2002), a nonparametric method for testing multivariate differences among pre-defined groups (groups = sampling date: 1968 or 2003).

Changes in species richness at regional scale were evaluated in both communities as the difference in their gamma diversity index between sampling dates. Gamma diversity (number of species in a community) is represented by all species present in all sites of each community type on each date. Considering that some species could have been missed during sampling, the first-order Jackknife index (Heltshe & Forrester 1983) was used to estimate the true number of species in a community (Eq. 3) and its variance (Eq. 4).

$$\hat{\gamma} = \gamma_{obs} + u\frac{n-1}{n} \tag{3}$$

$$Var(\hat{\gamma}) = \frac{n-1}{n}\left(\sum_{s=1}^{\gamma_{obs}} s^2 f_s - \frac{u^2}{n}\right) \tag{4}$$

where γ_{obs} is the observed species number in the n sites, u is the total number of unique species (recorded in only one site) and f_s is the number of sites containing s unique species. These estimators were used to define confidence intervals for gamma diversity (Heltshe & Forrester 1983) to determine statistical changes between both sampling dates.

To assess if compositional heterogeneity pattern (variation in species composition among sites) had been altered in 35 yr, we focus on two indicators: Whittaker's effective species turnover (Eq. 5; Whittaker 1972; Toumisto 2010) and the beta Raup-Crick indicator (β_{RC}; Chase et al. 2011). To determine statistical changes in species turnover among groups of samples of both dates, sampling variance (Eq. 6), 95% confidence limits and hypothesis test for β_{M-1} were calculated following Kiflawi & Spencer (2004).

$$\hat{\beta}_{M-1} = \frac{\hat{\gamma}}{\bar{\alpha}} - 1 \tag{5}$$

where $\bar{\alpha}$ is the mean number of species per site and $\hat{\gamma}$ the Jackknife estimator of gamma diversity

$$Var\left(\ln\left(\hat{\beta}_{M-1}\right)\right) \cong \left[\frac{\hat{\gamma}}{\bar{\alpha}(\hat{\gamma}-\bar{\alpha})}\right]^2 Var(\bar{\alpha}) + \left[\frac{1}{(\hat{\gamma}-\bar{\alpha})}\right]^2 Var(\hat{\gamma}) \tag{6}$$

The beta Raup-Crick indicator (β_{RC}) enables, using a null model approach, the assessment of whether variation in dissimilarity among sites derives from changes in the underlying structure or simply from differences in alpha or gamma diversity. The average β_{RC} between simultaneous samples within the same community type was calculated using the R code (Chase et al. 2011). It represents a mean dissimilarity index related to the null expectation, indicating whether each community type and sampling date are more dissimilar (approaching 1), as dissimilar (approaching 0) or less dissimilar (approaching -1) than expected by chance. Mean β_{RC} among sites will be negative when environmental filters shared across sites create highly similar communities. To assess whether heterogeneity between communities had changed we compared, between sampling dates, the proportion of common species in the whole species list of the MP and the HS.

The initial and final size of each group of species was determined for each community type and sampling date considering the whole list of recorded species to determine whether there was a significant change in origin and functional group composition. The number of species within each category, species that 'arrived' (present only in 2003), 'stayed' (present in 1968 and 2003) and 'disappeared' (absent in 2003), was also determined. We used the chi

square test to check statistical independence between plant functional types and the above-defined categories.

Results

Local analysis

In the MP, paired test results showed that mean richness per site increased between 1968 and 2003 ($\Delta \bar{\alpha}_i = 21$ species; $P < 0.0001$), whereas in the HS it remained constant ($\Delta \bar{\alpha}_i = 2$ species; $P = 0.3611$). Mean alpha diversity ± 1 SD in the MP was 46 ± 8.48 in 1968 and 67 ± 12.74 in 2003; in the HS they were 20 ± 5.96 and 22 ± 8.39, respectively. There is also evidence for consistency in the direction of change between sites in the MP, the greater part of sites showing increased species richness, but this pattern was not observed in the HS (Fig. 2).

Mean site colonization rate was higher ($P < 0.01$; Fig. 3a) and mean site extinction rate was lower ($P < 0.01$; Fig. 3b) in the MP than in the HS. In both communities new species (i.e. only detected in 2003) showed a lower mean site colonization rate than old species (already present in 1968) ($P < 0.0001$ in MP and $P = 0.002$ in HS; Fig. 3c and d), showing the increased ability of old species to spread across community sites. No significant differences were found between native and exotic mean colonization rates for these two communities ($P = 0.162$, $n = 29$ in MP and $P = 0.0764$, $n = 22$ in HS).
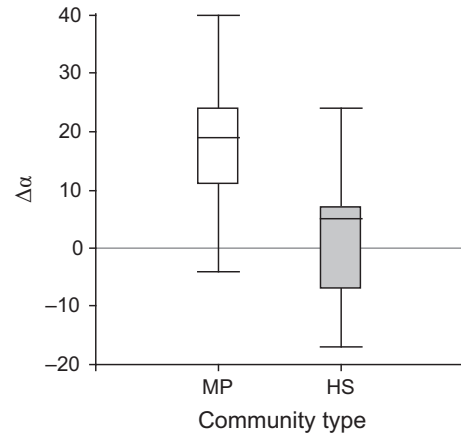

Fig. 2. Change in number of species per site between sampling dates for the mesophyte prairie (MP, $n = 29$) and the halophyte steppe (HS, $n = 22$).

By contrast, the extinction rate did not differ between natives and exotics in the MP ($P = 0.0548$, $n = 29$) but was significantly higher for exotic species in the HS (mean extinction rate: exotics = 0.82, natives = 0.46; $P < 0.0001$, $n = 22$).

While 36 species colonized more than 25% of the resurveyed sites of the MP (those species that colonized >50% are listed in Table 1), only three species colonized more
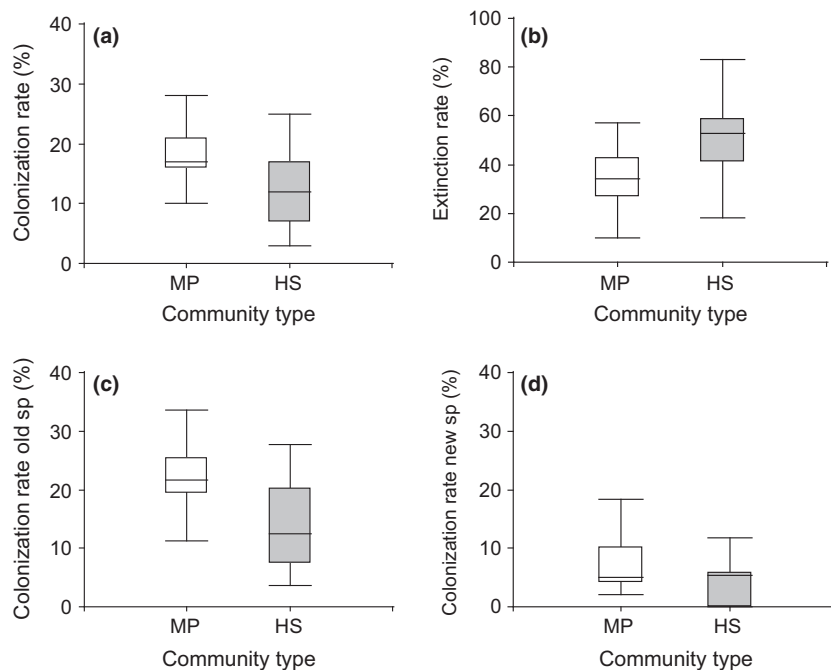

Fig. 3. Box-plots showing the distribution of (**a**) site colonization rate (a_i), (**b**) site extinction rate (e_i), (**c**) site colonization rate of old species (a_{1i}) and (**d**) site colonization rate of new species (a_{2i}) for the mesophyte prairie (MP) and the halophyte steppe (HS).

Table 1. Species that most increased or decreased their regional occupancy (as a proportion of presence in sites of a given community type) during the study period. CS, cool season; WS, warm season.

	Presence 1968	Presence 2003	Origin	Longevity	Growing season
Mesophytic prairie sites					
Species that increased					
Lotus glaber	0.14	0.93	Exotic	Perennial	WS
Hypochaeris radicata	0.31	0.86	Exotic	Perennial	CS
Mentha pulegium	0.17	0.76	Exotic	Perennial	CS
Baccharis trimera	0.03	0.76	Native	Perennial	CS
Cyperus eragrostis	0.00	0.69	Native	Perennial	WS
Medicago lupulina	0.10	0.62	Exotic	Annual	CS
Juncus microcephalus	0.00	0.55	Native	Perennial	CS
Solidago chilensis	0.00	0.52	Native	Perennial	WS
Species that decreased					
Margyricarpus pinnatus	0.59	0.31	Native	Perennial	CS
Chevreulia sarmentosa	0.52	0.14	Native	Perennial	CS
Wahlenbergia linarioides	0.48	0.17	Native	Perennial	CS
Eragrostis lugens	0.45	0.17	Native	Perennial	WS
Sporobolus platensis	0.31	0.00	Native	Perennial	WS
Halophytic steppe sites					
Species that increased					
Puccinellia glaucescens	0.29	0.55	Native	Perennial	CS
Lotus glaber	0.13	0.50	Exotic	Perennial	WS
Cyperus reflexus	0.21	0.45	Native	Perennial	CS
Species that decreased					
Sisyrinchium pratense	0.54	0.14	Native	Perennial	CS
Lepidium aletes	0.38	0.00	Native	Annual	CS
Agalinis communis	0.25	0.00	Native	Annual	WS

than 25% of the surveyed sites of the HS. The species that most increased their regional occupancy in both community types were natives and exotics, mostly perennials, and belong to groups from both growing seasons. In contrast, in both communities, the species that most reduced their regional occupancy were exclusively natives.

Site ordination with CA shows that the main pattern of between-site variability in species composition was associated with community type, with the first axis explaining 20.9% of total inertia (Fig. 4). The next principal axis represents temporal changes in site species composition, explaining 6.38% of total inertia. For both dates, distances on the ordination plane between the MP sites are shorter than between HS sites, showing higher homogeneity in species composition in the former. However comparing temporal distances in each community, changes were relatively more consistent in the MP than in the HS. The MRPP analysis of changes in site species origin and functional type composition showed significant differences between sampling dates in both communities (MP $P < 0.0001$; HS $P = 0.007$).

The comparison of paired temporal differences in proportional richness of origin and functional groups in MP sites showed a significant increase of exotic, warm-season and perennial species. The first two categories also increased in proportional cover (Table 2). In the HS sites,
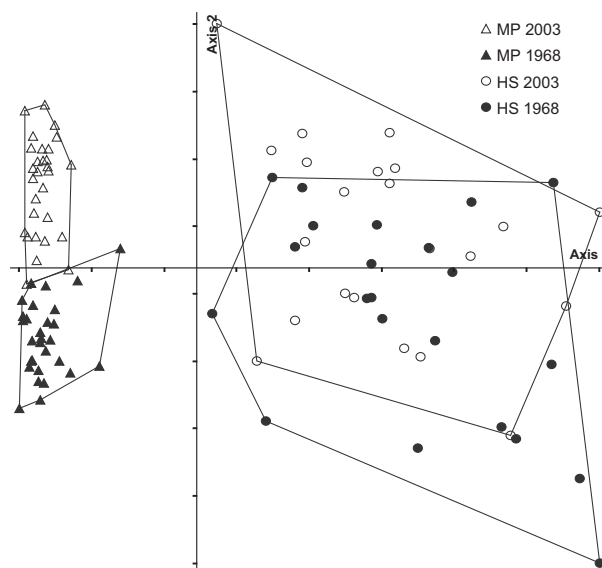
Fig. 4. Site ordination (CA) based on the species presence/absence matrix. Site symbols refer to community type and sampling date: ● halophyte steppe (HS) 1968, ○ halophyte steppe (HS) 2003, ▲ mesophyte prairie (MP) 1968, △ mesophyte prairie (MP) 2003.

warm-season and grass species increased in proportional richness and cover, while exotics increased in proportional cover but not in proportional richness.

Table 2. Proportion of species richness and cover of functional types and origin (mean ± SD) on two sampling dates. Different letters within species groups indicate significant differences between sampling dates at 5% (paired Wilcoxon test).

Functional type & origin	Sampling year	Mesophyte prairie		Halophyte steppe	
		Richness	Cover	Richness	Cover
Warm-season	1968	0.42 ± 0.06 a	0.45 ± 0.09 a	0.39 ± 0.14 a	0.50 ± 0.14 a
	2003	0.46 ± 0.03 b	0.52 ± 0.09 b	0.45 ± 0.10 b	0.61 ± 0.12 b
Perennials	1968	0.22 ± 0.04 a	0.82 ± 0.06 a	0.80 ± 0.09 a	0.86 ± 0.05 a
	2003	0.25 ± 0.05 b	0.83 ± 0.05 a	0.83 ± 0.09 a	0.89 ± 0.08 a
Grasses	1968	0.32 ± 0.05 a	0.48 ± 0.08 a	0.39 ± 0.09 a	0.51 ± 0.14 a
	2003	0.31 ± 0.04 a	0.51 ± 0.07 a	0.57 ± 0.08 b	0.73 ± 0.08 b
Exotics	1968	0.20 ± 0.06 a	0.18 ± 0.10 a	0.17 ± 0.08 a	0.15 ± 0.10 a
	2003	0.23 ± 0.05 b	0.25 ± 0.12 b	0.15 ± 0.09 a	0.25 ± 0.12 b

Table 3. Observed gamma diversity values, jackknife estimated values, SE and 95% confidence limits for the mesophyte prairie (MP) and the halophyte steppe (HS) on two sampling dates.

Community	Year	γ_{obs}	$\hat{\gamma}$	$S(\hat{\gamma})$	Confidence limits
MP	1968	163	203.6	8.9	185.8–221.4
	2003	209	253.0	9.3	234.4–271.6
HS	1968	98	138.0	8.9	120.2–155.8
	2003	91	122.5	7.8	106.9–138.1

Regional analysis

There was a significant increase in gamma diversity over time in the MP (Table 3), as the upper confidence limit for jackknife estimate value of gamma diversity in 1968 was smaller than the lower limit for the same index in 2003. In the HS no significant decrease was found, as confidence limits clearly overlapped.

Between sampling dates, we observed a significant decrease in species turnover (β_{M-1}) in the MP ($P = 0.02$; Table 4). By contrast, in the HS no clear evidence of biotic homogenization was found ($P = 0.06$). Indeed, sites of the HS had higher heterogeneity in species composition than those of the MP for both dates. We found that mean β_{RC} was negative among samples of each community type for both dates (Table 4), as expected for similar environmental filters shared across sites. On the other hand, in the MP mean β_{RC} between sites was lower in 2003 than in 1968, ensuring that the patterns we found with β_{M-1} were not

Table 4. Species turnover, P-values from hypothesis test and mean beta Raup-Crick between pairs of simultaneous sample sites for the mesophyte prairie (MP) and the halophyte steppe (HS) on two sampling dates.

Community	Year	$\hat{\beta}_{M-1}$	P-value	$\bar{\beta}_{RC}$
MP	1968	3.42	0.02	−0.41
	2003	2.78		−0.58
HS	1968	5.91	0.06	−0.33
	2003	4.65		−0.25

driven by the large increases in gamma and alpha diversity. The proportion of species shared by both community types decreased from 34% in 1968 to 26% in 2003. At the last sampling date, we recorded 176 exclusive species, 79 of them (45%) were already exclusive in 1968, 67 (38%) were newly arrived to the MP, 12 species (7%) were newly arrived to the HS and 18 species (10%) common to both communities disappeared from the HS.

In the MP we found significant dependence between species groups combining species origin and growing season with sampling date. Within the arrived species we distinguished new species to the community (absent in 1968) and old species (already present even in a site not resurveyed in 2003). The proportion of new warm-season exotic species (0.16) was significantly higher than that expected considering the proportion present in 1968 (0.037; $P = 0.002$; Table 5). The proportion of old exotic cold-season species that arrived at the resurveyed sites (0.36) was significantly higher than that expected considering their proportion in 1968 (0.14; $P = 0.005$; Table 5). No significant association was found for the other plant functional types.

Discussion

The grassland communities in the Flooding Pampa have changed over time, both in species richness and composition. Sites of the two community types showed very different transformation patterns of species diversity over the last 35 yr. Changes in richness were higher in the extended stands of the MP associated with deep, well-drained soils than in the HS composed of smaller patches and exposed to higher abiotic stress. Contrary to our prediction, regional richness in the MP increased 28%, while the average local diversity increased 46%. In the HS these changes were not significant. An analogous increase in regional richness and a slightly lower increase at local scale (37%) have been found in chalk grasslands during a time period twice as long as ours (Newton et al. 2012). Similar

Table 5. Number of species present in 1968 and arrived to the mesophyte prairie resampled sites, new species detected only in 2003, and old species already present in 1968 in other sites of the community. Species are classified by origin and growing season. *P*-values for statistical independence (chi square test).

Origin	Growing season	Present in 1968	New species	*P*-value	Old species	*P*-value
Native	Cold	65	11		9	
	Warm	69	21	0.149	8	0.99
Exotic	Cold	23	9	0.258	10	0.005
	Warm	6	8	0.002	1	0.970
Total		163	49	0.005	28	0.047

rises in local diversity were reported for environments with slight human intervention (Altesor et al. 1998; Sax & Gaines 2003). In contrast, for European countries local diversity decreased in association with similar low and intermediate levels of agricultural intensification (Flohre et al. 2011).

The colonization rate was higher and the extinction rate was lower in the richest community type (MP), in contrast to what would be expected under the community saturation model (Elmendorf & Harrison 2011). This is related to the large increase in local diversity observed in MP sites. The saturation process may be prevented in this environment by grazing pressure that avoids competitive exclusion through partial biomass removal. We were not able to find differences between natives and exotics in colonization rates in the MP, the community that showed more floristic changes. These results seem to support the idea that native and exotic species essentially function within communities following similar ecological rules (Meiners 2007). We must also take into account, as in Meiners' study, that most exotic species in the MP are not new arrivals but are largely naturalized within the system. In the HS sites, instead, where changes in richness and species composition are smaller and the number of naturalized exotic species is very low, we found higher significant extinction rate for exotics than for natives.

The first ordination axis clearly separates sites with greater abiotic constraints (HS) from those with less restriction (MP), regardless of inventory date. This shows the high importance of abiotic environmental conditions as drivers of species composition and richness in these grasslands (Perelman et al. 2001, 2007). The impact of differences in soil restrictions between community types overrides long-term changes in the same way as across spatial scales (Perelman et al. 2001). The current results appear to be consistent with the metacommunity concept of species sorting (Leibold et al. 2004), which is based on niche theory, where the presence and abundance of species in a community is determined by patch types or resource gradients. In the HS we found high heterogeneity values in species composition at both inventory dates. This can be interpreted as a consequence of the mass effect

process (Shmida & Wilson 1985), where species of surrounding areas can arrive to these sites and be recorded when sampling, without establishing long-term populations in this environment. This is also supported by the higher extinction rate of exotics in this community type. As a result, in the HS sites a series of different species from surrounding communities can be found accompanying the relatively stable set of species that are more competitive under such stress conditions. This coincides with the mass-effect perspective of metacommunity theory, which focuses on the role of immigration and emigration in the spatial dynamics of local population densities (Leibold et al. 2004).

The proportional contribution to richness of functional types in the regional flora did not show significant differences between sampling dates in life form (herbs vs grasses) nor in longevity (annuals vs perennials) as could be expected in these grasslands in response to grazing (Chaneton et al. 2002) and as reported at regional scale for North America (Quian & Guo 2010). Within the arrived species we only found significant differences in relation to initial proportions in species growing season and origin. Among new species to the community (absent in 1968), the proportion of warm-season exotic species was higher than that expected by chance (Table 5). This could be associated with the reported long-term increase in minimum temperature throughout the region, with significant decreases in the length of the frost-free period, the daily thermal amplitude and an increase in daily minimum temperature (Fernández-Long et al. 2013; for the period 1940–2007). Among the arrived old species (already present in the community but not in the resurveyed sites), the proportion of cold-season exotic species was higher than expected (Table 5). Most of the original invaders to these grasslands have been cold-season species (Perelman et al. 2007), and the increase we found would indicate an expansion of these species to other sites of the community instead of the arrival of new species.

At local scale, the increment in the proportion of warm-season species discussed above for the regional flora is also evident in both communities (Table 2). Although the same is true for exotics in the MP, in the HS their proportion

increased in cover and not in richness, in agreement with the observed higher extinction rate for exotics in this stressed environment. A few successful exotic species, like *Lotus glaber*, *Cynodon dactylon* and *Thinopyrum* sp., reach high cover values in the HS sites, while most exotics that arrive did not leave stable populations. At this scale, we also found significant increases in proportional richness of perennials in the MP and in the proportion of grass richness and cover in the HS (Table 2). Perennial species that expanded to new sites in the MP (>50% increase in constancy) and in the HS (only 20–35% increment) included both exotics and natives (Table 1). Grasses that increased their constancy and cover (1–5% increment) in the HS were *Paspalum vaginatum*, *Puccinellia glaucescens*, *Thinopyrum* sp. and *Cynodon dactylon,* encompassing both natives and exotics. Although the proportion of functional types showed changes in both communities, these were always of higher magnitude in the mesophyte prairie.

Biotic homogenization was evidenced in the MP as a significant reduction in species turnover and a decrease in mean beta Raup-Crick dissimilarity between sampling dates. In contrast to other findings (Olden & Rooney 2006; Quian & Guo 2010), the homogenization process in our study area was not promoted by exotic species invasion, but mainly by the spread of both native and exotic species already present in other sites of the same community ('old species'). A possible explanation is that in this region, land-use intensification has been more related to cattle and machinery movement carrying propagules, than to grassland replacement and fragmentation. In the Flooding Pampa, landscape fragmentation is the lowest among the subregions of the Pampa: the natural grasslands are still the predominant land-cover type (78.6%) characterized by a low level of dissection, in contrast to the most modified Rolling Pampa, where croplands cover 60.5% of the area (Baldi et al. 2006). In the HS community there is no evidence of biotic homogenization between sites, coinciding with smaller changes in species richness and composition observed in this more stressed environment. Between the two communities and considering the total list of species, no sign of homogenization was found. On the contrary, there are less common species between communities in the latest sampling date, due mainly to a regional increase in richness in the MP in species not shared with the HS. It seems that although we found more overlapping species among sites of the same community type, we are not facing a homogenization process that will lead to an impoverished flora and to a reduced regional distinctiveness.

In our study area, after 35 yr under moderate grazing intensity, we found evidence of an increase in species richness, no biodiversity loss and no losses of native species. Different grassland communities showed clear differences in changes of richness, in degree of homogenization and in species turnover. Exotic species increased only at local scale in the richest community type as a consequence of expansion of exotic species already present in the region and not to new arrivals. While great effort has gone into identifying the consequences of diversity losses (Naeem 2002; Bellard et al. 2012), little is known about the possible effect of increases in local diversity upon ecosystem functioning and community processes (Sax et al. 2002; Sax & Gaines 2003). Abiotically stressful environments are less prone to invasion than benign ones (Gerhardt & Collinge 2007), which explains the high extinction rate of exotic species in the HS community type. In addition, the alien flora that invaded these grasslands was mostly of Eurasian origin, particularly from arable soils in cropping environments of the Mediterranean region (Soyrinki 1991), lacking appropriate morphological and physiological traits required to tolerate frequent flooding and soil salinity, while having a predominance of species adapted to the MP environment.

Functional types varied considerably at local scale, but instead of those species favoured by grazing as expected, those that increased were warm-season species. These changes seem to be more associated with climate changes than disturbance intensity. These grasslands have been devoted to extensive grazing activities since the beginning of European colonization (Hall et al. 1991; Viglizzo et al. 2001) and have not changed much during the last decades: only 1.6% of grassland area in this region has been replaced by an alternative land cover (Baldi & Paruelo 2008). The extinction debt associated with anthropogenic disturbance by grazing may have already been paid.

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