Cattle change plant reproductive phenology, promoting community changes in a post-fire *Nothofagus* forest in northern Patagonia, Argentina

Manuel de Paz* and Estela Raffaele

Laboratorio Ecotono, INIBIOMA, CONICET-Universidad Nacional del Comahue, Quintral 1250, San Carlos de Bariloche, Rio Negro 8400, Argentina
*Correspondence address. Laboratorio Ecotono, INIBIOMA, CONICET-Universidad Nacional del Comahue, Quintral 1250, San Carlos de Bariloche, Rio Negro 8400, Argentina. Tel: +54-02944-423374; Fax: +54-02944-422111; E-mail: manolodpz@yahoo.com.ar.

Abstract

Aims

Variations in rates and length of flowering and fruiting not only affect the reproduction of a given plant species but also the behavior and reproduction of associated taxa. Flowering and fruiting variations may be influenced by herbivory, especially by large mammals. The aim of this study was to determine the effects of cattle browsing on the reproductive phenology of understory species in a subalpine post-fire *Nothofagus* forest in Patagonia.

Methods

The effects of herbivory on plant reproductive phenology were studied in a set of experimental exclosures (fenced plots) installed since 2001 in a post-fire *Nothofagus pumilio* forest, located in Nahuel Huapi National Park (NHNP), Argentina. We monitored the beginning and duration of each reproductive phenological stage: floral bud, open flower, immature fruit and mature fruit. We also counted the number of flowers, fruits, seeds and viable seeds of the dominant plants to assess whether browsing modifies temporal patterns of the flowering and fruiting periods.

Important Findings

Cattle reduced the total number of species flowering and fruiting and changed the reproductive phenology of some species. We found that palatable species seem to be negatively affected by browsing in terms of reduced fitness due to changes in flowering and fruiting periods. In contrast, cattle benefitted the reproduction of non-palatable species and could promote the invasion of shade-intolerant exotic forbs such as *Cirsium vulgare*. The effects of livestock reported in this study are important to understanding how browsing could alter native species establishment and possibly alter successional trajectories during recolonization after fire.

Keywords: plant fitness, livestock, browsing, flowering, fruiting

Received: 26 July 2012, Revised: 29 December 2012, Accepted: 5 January 2013.

INTRODUCTION

Plant reproductive phenology plays an essential role in terrestrial ecosystems (Herrera 1986). Variations in duration and rates of flowering and fruiting not only affect the reproduction of plant species but also the behavior and reproduction of other taxa, e.g. animals that depend directly or indirectly on plant phenologies (Aizen 2007; Aizen and Feinsinger 1994; Smith-Ramírez *et al.* 1998). Causes of these variations are diverse and widely studied (Lehtilä and Strauss 1997; Paritsis *et al.* 2006; Smith-Ramírez *et al.* 1998). The most common factors affecting variations of flowering and fruiting phenology are changes in temperature (Arroyo *et al.* 1981; Menzel *et al.* 2006), photoperiod (Henrich 1976), precipitation (Opler *et al.* 1980), light availability, humidity and soil nutrient contents (Paritsis *et al.* 2006; Smith-Ramírez *et al.* 1998). Furthermore, flowering and fruiting variations may be influenced by herbivory (Ivey and Carr 2005; Lehtilä and Strauss 1999), especially by large mammals such as cattle, e.g. *Bos Taurus* L., and horses, e.g. *Equus ferus caballus*

L. (Vázquez and Simberloff 2003, 2004). Herbivory may change plant community as a result of biomass removal, soil compaction and/or indirect effects of altering total plant cover and species composition. However, the activities of herbivores can also affect plant growth, architecture, chemistry and phenology, which in turn may affect insect interactions (Gómez and González-Megías 2007; Juenger and Bergelson 1998; Sasal 2009; Vázquez and Simberloff 2004) and plant reproduction (Brown *et al.* 1987; Lehtilä and Strauss 1997; Mothershead and Marquis 2000; Poveda *et al.* 2003). Also, the introduction of exotic herbivores has been shown to have important influences on the micro-environmental conditions (e.g. Tercero-Bucardo *et al.* 2007). There has been some recent evidence implying that introduced vertebrate herbivores may contribute to the invasiveness of exotic plants, in agreement with the invasional meltdown hypothesis (Nuñez *et al.* 2010).

An upsurge in large, severe fires in the late 20th and early 21st centuries in northern Patagonia (Veblen *et al.* 2011), as well as the introduction of exotic plants and large herbivores (Vázquez and Simberloff 2003; Veblen *et al.* 1992a, 2005), has produced important ecological modifications in northwestern Patagonian forests. Exotic herbivores have shown important influences on the floristic composition and structure of a wide range of community types (Kitzberger *et al.* 2005; Relva and Veblen 1998; Vázquez 2002; Veblen *et al.* 1989, 1992a) as well as the possible modification of the habitat, which can have an impact on plant–pollinators' and plant–seed dispersers' interactions (Vázquez and Simberloff 2003). Cattle can modify soil properties (e.g. increase soil compaction, nutrient input), environmental heterogeneity and increase defoliation of plants (Sasal 2009). Our previous works have elucidated consistent patterns of post-fire vegetation changes, both in the presence and absence of introduced herbivores (Blackhall *et al.* 2008; Kitzberger and Veblen 1999; Raffaele and Veblen 1998, 2001; Raffaele *et al.* 2011; Tercero-Bucardo *et al.* 2007; Veblen and Lorenz 1987; Veblen *et al.* 1992a). In that context, the goal of this study is to identify the mechanisms by which introduced herbivores change the reproductive phenology of the dominant species of native forests and shrublands in northern Patagonia. In contrast to the abundant research identifying the effects of exotic herbivores on the vegetation and mechanisms by which livestock alter the structure and composition, sometimes creating conditions that favor the perpetuation of alien plants (Brooks *et al.* 2004), the role of introduced herbivores in altering the fitness and reproductive phenology of the species has received little research attention. This kind of information may contribute to the understanding of other potential browsing effects on native communities, which could be especially important during community regeneration after disturbance (Blackhall *et al.* 2008).

In a post-fire deciduous *Nothofagus* forest, we analyzed different browsing effects (direct and indirect) on the reproductive phenology of some key native shrubs with different palatability to browsing of livestock and a common exotic herb species (*Cirsium vulgare*), commonly associated with livestock and fire.

We expected negative direct effects (e.g. low flowering) to be stronger on palatable species due to the browsing effect (e.g. removal of plant biomass). In contrast, the indirect effects (e.g. on timing of flowering and fruiting) would be expected to be stronger on the unpalatable species, because cattle indirectly change environmental resources (e.g. high light) and increase microsite availability for these species. This study reports the effects of cattle browsing on reproductive plant phenology. Thus, the primary aim of this work is to determine if there are any differences in the flowering and fruiting phenology of the dominant species, comparing plots with the presence and absence of livestock in a post-fire *Nothofagus* forest. In the current research, we also address the following questions: (1) Does browsing modify the period of reproductive phenology of native and exotic plants? (2) Does the extent of the livestock effects vary according to the species palatability?

METHODS

Experimental site

This study was conducted in a *Nothofagus pumilio* (Poepp & Endl) Krasser forest, severely burned in January of 1999. It is located in Donat Hill (41°20′ S, 71°00′ W, 1250 m), NHNP, Argentina. This type of forest characterizes the subalpine zone from 1000 to 1100 m.a.s.l. to the upper tree line at ca. 1450 m.a.s.l. The site has a temperate humid climate with a mean precipitation of approximately 1700 mm per year, occurring mainly from April to September as rain and snow. In this region, the precipitation abruptly decreases from 4000 mm per year on the west Andes to 500 mm per year only 80 km to the east (Veblen *et al.* 1992b, 2003).This area shows a long history of cattle browsing and spontaneous incidence of the European hare (*Lepus europeus* Pallas) before fire (Raffaele *et al.* 2011). Cattle occurred in moderate numbers at our sample sites for at least 30 years prior to the 1999 fire. Thus, the sites fit the common pattern of livestock use of *N. pumilio* forests in the national reserve zone of NHNP.

We studied the phenological responses of 12 plant species to cattle in a set of experimental exclosures (fenced plots) installed since 2001. This experiment is part of a network of long-term experiments of cattle exclusion located in different types of forest in the NHNP and Lanín National Park. Eight years after the last fire (1999), the dominant woody species were the following shrubs: *Berberis microphylla* G. Forst., *B. darwinii* Hooker, *B. serrato-dentata* Larcher, *Maytenus chubutensis* (Speg.) Lourt, *Ribes magellanicum* Poir, *Baccharis obovata* Hooker et Arnot, and *Schinus patagonicus* (Phil.) Jonnst. The most abundant climbers were *Vicia nigricans* Hooker et Arnot and *Mutisia spinosa* Ruiz & Pav. One species of bamboo (*Chusquea culeou* E. Desv.) is also present. In addition to the native plants, the common exotic forbs include *Circium vulgare* L. and *Carduus nutans* (Savi) Ten. All these species resprout after fire (Raffaele and Veblen 2001; Veblen *et al.* 2003), except *V. nigricans* and the exotic forbs that regenerate from seed banks (Ghermandi *et al.* 2004; Silvertown and Smith

1989). The following species are considered palatable plants (frequently browsed by cattle): *R. magellanicum*, *S. patagonicus*, *Ba. obovata*, *M. chubutensis*, *M. spinosa* and *V. nigricans*, and non-palatable species (low browsing) are *B. microphylla*, *B. darwinii*, *B.serrato-dentata*, *C. vulgare*, *Cs. nutans* and *Rosa rubiginosa* L. (Blackhall 2006; Blackhall *et al.* 2008; Raffaele *et al.* 2011).

Experimental design

The effects of herbivory on plant reproductive phenology were studied on individual plants and at the community level. At both scales, we monitored the duration of each reproductive phenological stage: floral bud, open flower, immature fruit and mature fruit (see Paritsis *et al.* 2006 for details). We specifically examined the following components of flowering and fruiting phenology: (1) timing of first flowering and fruiting, (2) duration of flowering and fruiting, (3) flowering and fruiting patterns, i.e. how the rate of flower and fruit productions vary over the phenological period, and (4) overlap in flowering and fruiting among species ('synchrony').

On the experimental site, five cattle exclosures of 25×25 m were installed in 2001 with their associated unfenced controls, following a paired plot experimental design (Sokal and Rohlf 1969). On each one of the five blocks (pair exclosure-control), five individuals of *R. magellanicum*, *B. microphylla* and *C. vulgare* were selected and marked for the recording of the number of flowers, percentage of fruiting (fruit set) and number and viability of seeds. These three species were selected because they are the most frequent species in the plots, their covers being 5.27 % and 3.24% for the woody species, respectively, and 13.08% for the exotic herb (Table 1; Raffaele *et al.* 2011). During the 2006–7 growing season, from the third week of October to the first week of April, on each of the marked

individuals, three branches of similar size were selected by chance based on a preset rule for randomization and marked. From each branch, we registered the number of flowers and fruits throughout the reproductive stage. In addition, we measured in *C. vulgare* the widest diameter of the inflorescences as an indirect estimation of the number of flowers and fruits.

We collected fruits from marked individuals to estimate the number of seeds per fruit and the seed viability for each species. We estimated the fruit set as the percentage of open flowers that developed into mature fruits in the marked individuals. The number of seeds per fruit was estimated as the average of the number of seeds from five fruits collected from marked individuals. In the case of *C. vulgare*, the number of seeds per fruit was considered as the number of seeds per inflorescence. A total of 300 seeds per species were analyzed to estimate the seed viability by using indirect estimations, e.g. flotation in water, the hardness of the seeds and the physical aspects (e.g. health of the seed coat or tegument; Forcella *et al.* 2004). A subset of seed from the studied sample was randomly selected, dissected and observed under microscope to verify embryo viability.

At a community level, we studied the length and initial time of flowering and fruiting of the most frequent species in the study area during the early regeneration (*V. nigricans*, *C. vulgare*, *Cs. nutans*, *R. magellanicum*, *B. microphylla*, *B. darwinii*, *B. serrato-dentata*, *M. chubutensis*, *Ba. obovata*, *S. patagonicus*, *M. spinosa* and *R. rubiginosa*) inside the fenced and unfenced plots. Table 1 presents a brief description of basic biology of the 12 studied species (e.g. type of regeneration, life forms, palatability). We recorded weekly the phenological stage of the individuals from each woody species and the dominant forbs and grass species in two transects of 5×25 m located in

Table 1: reproductive characteristics, life forms, palatability and cover of the most frequent species in the study area

Species	Family	Life form	Resprouting capacity	Seed recruit	Seedbank	Palatability	Cover (%)
Baccharis obovata	Asteraceae	Perennial shrub	Basal buds	n.i.		High	1
Berberis darwinii	Berberidaceae	Perennial shrub	Basal buds	Low		Low	2.4
Berberis microphylla	Berberidaceae	Perennial shrub	Basal buds	Low		Low	3.2
Berberis serrato-dentata	Berberidaceae	Perennial shrub	Basal buds	n. i.		Low	1.9
Carduus nutans[a]	Asteraceae	Bianual forb	No	High	×	Low	3.9
Cirsium vulgare[a]	Asteraceae	Bianual forb	No	High	×	Low	13.1
Maytenus chubutensis	Celastraceae	Perennial shrub	Basal buds	n.i.		High	1.6
Mutisia spinosa	Asteraceae	Semi-woody climber	Yes	Low		High	1.6
Ribes magellanicum	Saxifragaceae	Deciduous shrubs	Yes	Low	×	High	5.3
Rosa rubiginosa	Rosaceae	Deciduous shrubs	Basal buds	Low	×	High	1
Schinus patagonicus	Anarcadiaceae	Perennial shrub	Basal roots	Low		High	1.9
Vicia nigricans	Fabaceae	Annual herb climber	No	Low	×	High	7

[a]Non-native species.
n.i. = no information.
Source: Blackhall *et al.* (2008), Cavallero (2012), Correa (1976), Varela *et al.* (2006), Veblen *et al.* (2003).

each fenced and unfenced plot. In addition, we registered the date when the first open flowers or mature fruits appeared on all the individuals and also recorded the number of species in a specific phenological state in each transect (Paritsis *et al.* 2006).

To assess the effectiveness of browsing effects by plant species, we used the browsing rates estimated in the field in previous studies (Blackhall *et al.* 2008; Raffaele *et al.* 2011).

Data analysis

An analysis of variance (ANOVA; Zar 1996) was carried out to test the cattle effects on plant reproductive output. The dependent variables were the following: the number of flowers produced per species, the percentage of viable seeds, and the seed set (numbers of seeds per fruit). Normality of residuals was evaluated using Shapiro-Wilk's test, and homogeneity of variances was assessed using Levene's test (Quinn and Keough 2002). When necessary, logarithmic and arcsine transformations were applied to improve normality and homogeneity of variances. Each variable was obtained from the mean of the three branches selected per individual. In *R. magellanicum* and *B. microphylla* the height of shrubs was used as covariable to control possible significant differences due to individual size. Two-way ANOVAs were performed to assess the effects of cattle on fruit production and between species, cattle (C) and species (S) being the two factors.

The phenological stages were recorded considering the first day of sampling as day one, the second day as day two, etc. The stages defined were floral bud, open flower, immature fruit and mature fruit (Paritsis *et al.* 2006). In this way, we estimated the beginning and duration (days) of the mentioned stages of flowering and fruiting for each species.

The effect of cattle on the duration of phenophases was analyzed per species by using one-way ANOVAs. The dependent variable is the average of days in each phenological stage per species on each transect. In order to assess the effect of herbivory on the flowering and fruiting onset and span per species, one-way ANOVAs were performed. Cattle were considered the main factor. On the plants recorded in the transects, located inside and outside fenced plots, flowering and fruiting onset were estimated as the number of days from the first sampling (day 1) to the appearance of the first flower and the first fruit, respectively. In all the cases, the ANOVA assumptions were tested and, when necessary, the variables were transformed or non-parametric tests were performed (Conover 1980). Response variable means are reported with standard errors (means ± SE).

We compared flowering and fruiting periods of palatable and non-palatable group species growing inside and outside the exclosures by using a priori contrast tests (Sokal and Rohlf 1969). For the estimation of number of flowers and fruits on *C. vulgare*, a previous index was validated through a random sampling of 25 inflorescences. The diameter of each inflorescence was measured and the number of flowers and fruits were counted. A linear regression analysis was performed,

detecting a high and positive relationship between inflorescence diameter and the number of flowers/fruits. These results allowed us to simplify the sampling effort because we estimated the number of flowers/fruits of the *C. vulgare* individuals by using the diameter of their inflorescences.

RESULTS

Number of flowers

Cattle significantly affected the number of flowers in the studied native woody species in different ways. In *R. magellanicum*, cattle negatively affected the number of flowers (255 ± 31 flowers per branch in fenced and 14 ± 5 in unfenced plots, $F_{1,4} = 26.85$, $P < 0.05$; Table 2, Fig. 1a), whereas *B. microphylla* significantly increased its production of flowers in the unfenced plots (3.6 ± 0.4 flowers per branch) with respect to the fenced plots (0.8 ± 0.3 flowers per branch, $F_{1,4} = 13.36$, $P < 0.01$; Table 2, Fig. 1a). There was no significant difference in the production of flowers of *C. vulgare* between fenced and unfenced plots ($F_{1,4} = 2.20$, $P > 0.05$; Table 2, Fig. 1a). The number of flowers of *C. vulgare* was positively related to inflorescence diameter (Diameter vs. Flowers: $Y = 1007.2 + 0.5X$; $R^2 = 50.6$, $P < 0.01$).

Fruit set and seed set

The effects of cattle on the percentage of fruits varied among the studied species (C × S: $F_{1,140} = 5.19$, $P < 0.05$). The fruit production calculated as fruit set (%) in *R. magellanicum* (palatable species) was significantly higher in the fenced plots (18%, 49.6 ± 5.7 fruits per branch) than in the unfenced plots (1.7%, 0.3 ± 0.2 fruits per branch; $F_{1,140} = 4.53$, $P = 0.03$; Fig. 1b). Conversely, *B. microphylla* (unpalatable species) significantly increased the number of fruits in unfenced plots (28%, 1.4 ± 0.27 fruits per branch) with respect to fenced plots (14%, 0.5 ± 0.2 fruits per branch; $F_{1,140} = 4.26$, $P = 0.04$; Fig. 1b). The number of fruits of *C. vulgare* was similar in the fenced plots (56 ± 6 fruits per inflorescence) with respect to the unfenced plots (61 ± 5 fruits per inflorescence; $F_{1,140} = 2.28$, $P = 0.13$). The number of fruits of *C. vulgare*

Table 2: ANOVA results for the effect of cattle on the number of flowers of *R. magellanicum*, *B. microphylla* and *C. vulgare*

Species	Source	df	MS	F	P
R. magellanicum	Cattle	1	723043.22	26.85	<0.01*
	Block	4	40630.92	1.51	0.22
	Error	44	26925.63		
B. microphylla	Cattle	1	97.07	13.36	<0.01*
	Block	4	13.16	1.81	0.14
	Error	44	7.26		
C. vulgare	Cattle	1	2462.6	2.20	0.14
	Block	4	2769.31	2.48	0.06
	Error	44	1120.01		

*Significant results are indicated with $P < 0.05$.
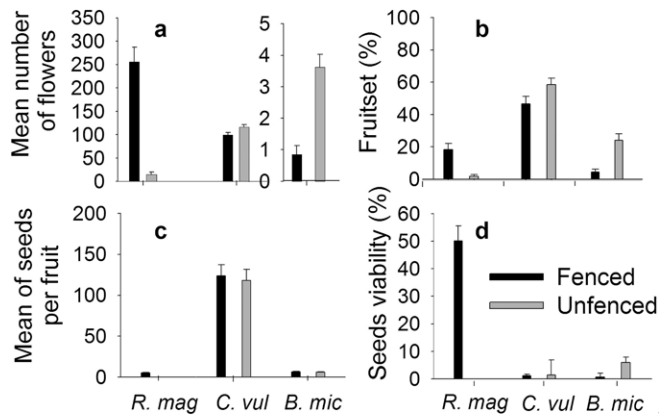
Figure 1: mean number of flowers (**a**), fruit set: percentage of open flowers that developed in ripe fruits (**b**), number of seeds per fruit (**c**) and viability of seeds (**d**) of the native woody species, *R. magellanicum* (*R. mag*) and *B. microphylla* (*B. mic*), and the exotic forb, *C. vulgare* (*C. vul*). Data from unfenced plots are not shown for *R. magellanicum* (**d**) because fruits with seeds are not recorded for this species.

was positively related to inflorescence diameter ($Y = 79.63 + 0.34X$; $R^2 = 34.2$, $P < 0.01$).

The mean number of seeds per fruit obtained from *B. microphylla* (6 ± 0.44 seeds per fruit for fenced plots, and 6.5 ± 0.4 for unfenced plots) and *C. vulgare* (118 ± 13.4 seeds per inflorescence for fenced plots, and 123 ± 13.2 for unfenced plots) was not significantly affected by cattle ($F_{1,44} = 3.06$, $P = 0.15$; $F_{1,44} = 0.31$, $P = 0.61$; Fig. 1c). The number of seeds produced per fruit in *R. magellanicum* was not estimated in unfenced plots because of the reduced number of fruits produced by this species. The mean production of seeds per fruit of this species is 7 ± 1.7 inside the fenced plots.

Seed viability

The seed viability of *R. magellanicum* was greater in the fenced plots than in the unfenced plots. This difference was so strong that we did not record any viable seeds in unfenced plots. Consequently, we were unable to statistically analyze this variable. Although the viability of seeds in *B. microphylla* tended to be higher in the unfenced plots than in the fenced plots (Fig. 1d), we did not find significant differences ($F_{1,44} = 4.54$, $P = 0.10$). In the case of *C. vulgare*, the seed viability was similar between fenced and unfenced plots ($F_{1,44} = 0.86$, $P = 0.41$; Fig. 1d).

Effect of cattle on synchrony of the flowering and fruiting

The total number of species flowering and fruiting was smaller in unfenced plots than in fenced plots during the entire growing season (Fig. 2a and b). Cattle also modified the distribution pattern of flowering species throughout this time. In fenced areas, we registered that the majority of species bloomed during January. Nevertheless, in unfenced plots, the distribution of species flowering was different. We recorded

two peaks of flowering during the growing season, the first in November and the second in February (Fig. 2b). In both fenced and unfenced plots, the amount of species fruiting began in January and increased with time. Exceptionally, in the unfenced plots, during March we observed a diminution in the number of species with fruits (Fig. 2b).

Flowering onset and flowering span

In general, the total reproductive period of all species lasted approximately 6 months. We observed a shorter flowering span in unfenced plots than in fenced plots (7.8 ± 1.6 and 15 ± 3.3, respectively; Fig. 3a). Independent of the presence of cattle, the first species to bloom were *Berberis* spp. and the last to maintain opened flowers were *V. nigricans* and *Cs. nutans*, whereas the last to flower was *M. spinosa* (Fig. 3a). The flowering onset was not affected by the cattle for any species ($F_{1,44}$, $P > 0.05$; Fig. 3a). Fifty percent of the studied species initiated their flowering in October with a peak in November. Changes in the phenological stages depended on the palatability of the species. The phenological stages of palatable species and non-palatable species showed significant differences in the temporal phenology patterns. In the unfenced plots, the flowering time of the palatable species was shorter (3 ± 2 days) than in the fenced plots (19 ± 5 days; Fig. 4a; $F_{1,113} = 5.63$, $P = 0.02$). *Baccharis obovata*, *R. rubiginosa* and *M. chubutensis* did not bloom in the unfenced plots, and *M. spinosa* did not bloom in fenced plots (Fig. 2a). The length of flowering time in the non-palatable species was not affected by cattle (10 ± 6 days for fenced and 12 ± 2 days for unfenced plots, respectively; Fig. 4a; $F_{1,113} = 1.08$, $P = 0.30$). In general, the length of the phenological stages in palatable species tended to be, on average, shorter in unfenced than in fenced plots, except for *S. patagonicus* (Fig. 3a). Nevertheless,
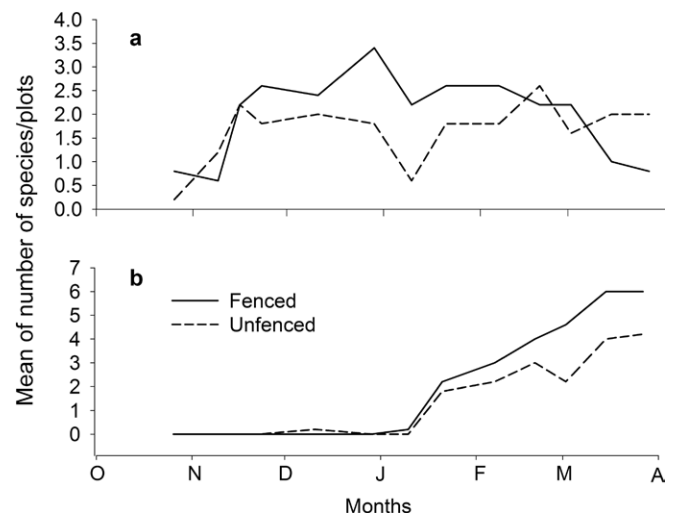
Figure 2: mean number of species recorded during the flowering period (**a**) and the fruiting period (**b**). Sampling time is recorded during the following months: O = October, N = November, D = December, J = January, F = February, M = March and A = April. The solid line corresponds to the fenced plots and the dotted line to unfenced plots.
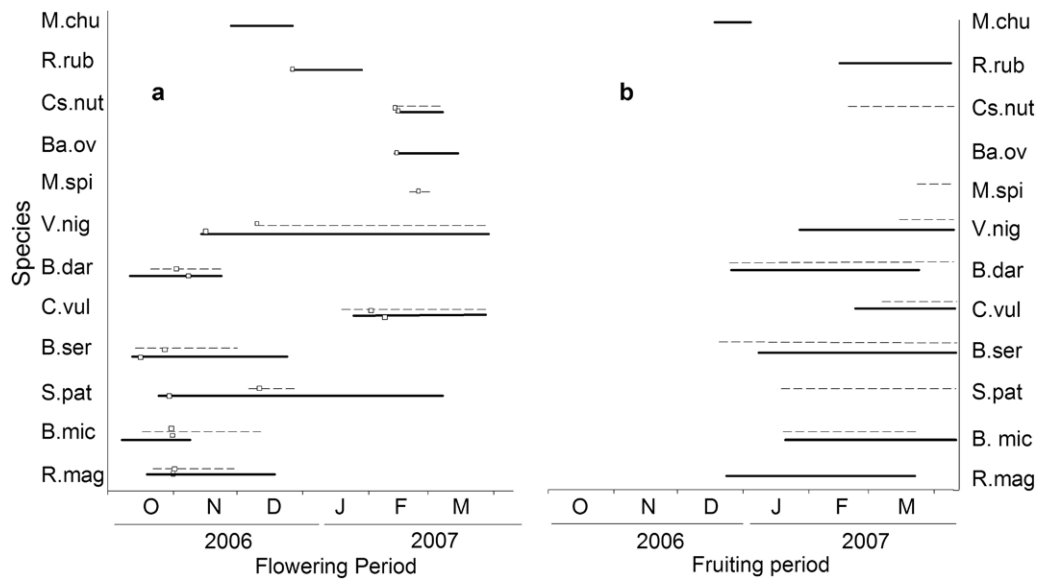
Figure 3: flowering and fruiting span of the most abundant woody species and forbs. The solid line indicates fenced plots (without cattle) and dotted line unfenced (with cattle). The white square indicates the flowering peak of each species. *R. mag* (*Ribes magellanicum*), *B. mic* (*Berberis microphylla*), *S. pat* (*Schinus patagonicus*), *B. serr* (*B. serrato-dentata*), *C. vul* (*Cirsium vulgare*), *B. dar* (*B. darwinii*), *V. nig* (*V. nigricans*), *M. spi* (*Mutisia spinosa*), *Ba. ov* (*Baccharis obovata*), *Cs. nut* (*Carduus nutans*), *R. rub* (*Rosa rubiginosa*). The species not included in (**b**) did not produce fruits.
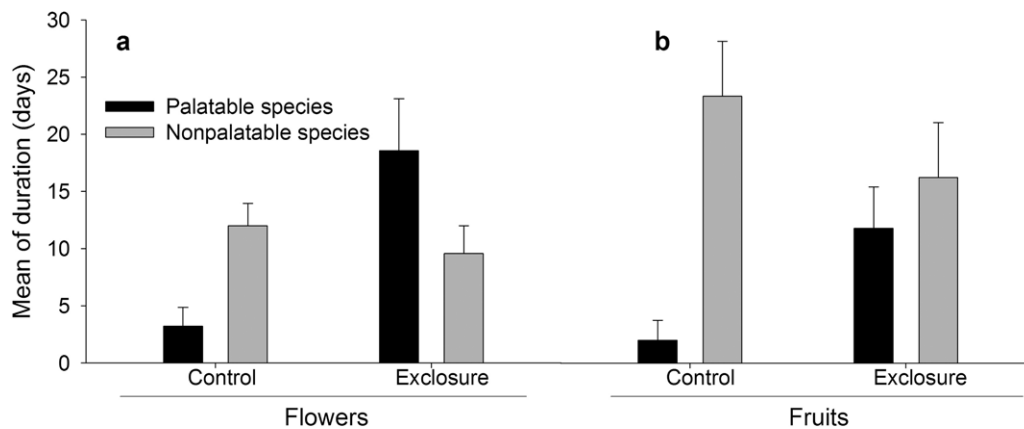
Figure 4: mean of flowering (**a**) and fruiting (**b**) periods of the palatable and non-palatable species growing in the fenced and unfenced plots, expressed in days. Bars indicate the standard errors.

this tendency was only significant in *R. magellanicum* during the stage of floral bud ($F_{1, 44} = 8.14$, $P = 0.04$; Fig. 3a). In contrast, the flowering periods of non-palatable species were, on average, longer in the unfenced than in the fenced plots (Fig. 4a), this tendency only being significant in *B. microphylla* ($F_{1,4} = 15.35$, $P = 0.02$), *C. vulgare* ($F_{1,4} = 14.88$, $P = 0.02$) and *B. darwinii* ($F_{1,4} = 7.87$, $P = 0.05$). Due to the lack of data from the flowering stages of *M. chubutensis*, *R. rubiginosa, M. spinosa* and *Ba. obovata* in the unfenced plots, statistical analyses were not performed (Fig. 3a).

Fruiting onset and fruiting span

The average time of fruiting was similar in unfenced and fenced plots (14 ± 3.4 days and 16 ± 3.7 days, respectively).

Berberis serrato-dentata was the first species fruiting in the fenced plots followed by *R. magellanicum* and *M. chubutensis* in unfenced plots during the second week of December (Fig. 3b). *Mutisia spinosa* in unfenced and fenced plots and *R. magellanicum* in unfenced plots did not produce fruits. In general, we observed fruits till the end of the growing season (April). An exception was *B. microphylla* in unfenced plots and *B. darwinii* and *R. magellanicum* in fenced plots, in which the fruits ripened before the last date of sampling (April 4; Fig. 3b). Most of the species did not show temporary changes in fruiting due to the browsing of cattle. However, *B. microphylla* fructified significantly earlier in unfenced plots (37 ± 5 days earlier; $F_{1,44} = 155.9$, $P < 0.01$) and *R. magellanicum* only produced fruits in fenced plots. In the palatable species group, the fruiting

span was 3 ± 2.4 days in unfenced plots and 17 ± 4.8 days in fenced plots (Fig. 4b; $F_{1,113} = 2.62$, $P = 0.1$). *Ribes magellanicum*, *M. chubutensis* and *R. rubiginosa* did not fruit in unfenced plots (Fig. 3b). For example, only two individuals of *R. magellanicum* produced fruits in all of the study area. Finally, the fruiting period of *V. nigricans* was significantly shorter in unfenced plots ($F_{1,44} = 12$, $P = 0.03$). In the case of the non-palatable species, there were no significant cattle effects on the fruiting length ($F_{1,113} = 0.54$, $P = 0.46$), being 23 ± 5.8 days in the unfenced and 16 ± 5.9 days in the fenced plots (Fig. 4b).

DISCUSSION

Our results showed strong evidence that cattle produce diverse effects on plant reproduction and that these changes mainly depend on the palatability of each plant species. A main result of our study is the drastic reduction in the production of flowers and fruits of the palatable species *R. magellanicum* (25 times fewer flowers, 10 times fewer fruits, null seed viability in the unfenced plots) due to cattle browsing. Probably, in the short term, browsing could reduce the sexual regeneration of this species. Similar results are found in the palatable species *Oenothera macrocarpa* Nutt. (Onagraceae) in Missouri grassland communities (Mothershead and Marquis 2000) and *Plantago maritima* L. in a salt marsh on Dutch sandy barrier (Dormann and Bakker 2000). However, in the long term, *R. magellanicum* may remain by resprouting, when suffering a high browsing pressure with low or null sexual reproduction. In fact, this phenomenon was observed in most of the individuals growing in unfenced plots, suggesting that this palatable species could be threatened by browsing not only by the elimination of biomass but also by the reduction in the production of flowers, fruits (strong and negative direct effect) and viable seeds. In contrast, cattle could benefit, as an indirect effect, the reproduction in non-palatable species. For example, the non-palatable species *B. microphylla* in the presence of cattle produced four times more flowers, two times more fruits and nine times more viable seeds than in fenced plots, increasing its reproduction. As a consequence, livestock can affect the diversity of plant communities, determining which plant species will be dominant. Similar results were observed in a post-fire shrubland located near our study site (Paritsis *et al.* 2006) and in a post-fire *N. dombeyi* forest (Sasal 2009).

The fruit set of *C. vulgare* was four and five times higher compared to *B. microphylla* and *R. magellanicum*, respectively. Although cattle did not significantly affect its fitness, its flowering period was longer in the unfenced than in the fenced plots, being particularly profuse in the presence of cattle (mean cover of 5.6% in fenced and 10.5% in unfenced plots, unpublished data). These results indicate that *C. vulgare* has a great competitive advantage among the studied native species. Therefore, this alien species could be a potential invader in the study area and provide abundant dry leaves at the end of the growing season. Thus, the relatively high cover of these introduced forbs as continuous fine fuel, as well as their phenology, may promote

vegetation flammability (Raffaele *et al.* 2011), as also reported in other ecosystems (Billings 1990; D'Antonio *et al.* 2000).

The high synchrony among species can favor the effective pollination of the species that bloom in the middle of the season (Osada *et al.* 2003). Another effect of cattle on the reproductive phenology recorded in this study is the low number of species that synchronize in different phenophases (flower and mature fruit). We found one peak of the mean number of all species flowering in fenced plots during January and two peaks in the unfenced plots. Cattle browsing specifically induced asynchrony in flowering and fruiting periods among individuals of the palatable species that flourished in the unfenced plots (*R. magellanicum*), and as the strongest browsing effect, there were three species (*Ba. obovata*, *R. rubiginosa* and *M. chubutensis*) that did not produce flowers in the presence of cattle. Similar effects are found in other browsing studies, which show that in the reproductive periods the livestock can alter the onset and the flowering span (Gómez 1996; Poveda *et al.* 2003; Verboom *et al.* 2002). We observed significant changes in the flowering span in only one palatable species (*R. magellanicum*), with flowers opening first in fenced plots. In contrast, flowering periods of the non-palatable species, *B. microphylla* and *B. darwinii*, and the exotic forb, *C. vulgare*, were extended by cattle browsing effects. These changes in flowering periods could alter the relationships with pollinators that affect the fitness of the plants. The long exposure of flowers to pollinators has been shown to increase the rate of removal and pollen deposition (Abdala-Roberts *et al.* 2007; Aximoff and Freitas 2010; Blair and Wolfe 2007). A recent study (Sasal 2009) found that cattle increased the frequency of pollinator visits and the fruit set on some woody non-palatable species (e.g. *B. darwinii*) in an early post-fire *Nothofagus* forest. The non-palatable species, e.g. *B. microphylla* and *C. vulgare*, showed an earlier flowering onset and duration in the unfenced plots compared to the fenced plots. A possible explanation could be that in the early post-fire succession both species are shade-intolerant (Damascos and Rapoport 2002). In this case, the non-palatable species would be indirectly favored in most open areas affected by browsing (Blackhall *et al.* 2008; Raffaele and Veblen 2001; Veblen *et al.* 2003).

CONCLUSIONS

Introduced ungulate browsing had varied effects on plant reproduction, and these effects varied in magnitude and direction depending on the palatability of the species. Some species that are highly browsed could experience a local extinction by reduction in the number of flowers, fruit set, seed set, viability of seeds, and by the alteration of their phenological periods. These drastic changes in the medium term would cause changes in plant composition or species dominance. We believe that eventually cattle could remove the fire-resilient palatable shrubs and with them the possibility of rapid post-fire regeneration by resprouting shrubs, which act as nurse species. Loss of these shrub species could also

reduce biotic resistance (sensu Elton 1958) of native plant communities to the establishment of exotic species (Nuñez and Raffaele 2007; Raffaele and Veblen 1998).

Another important aspect of ungulate herbivory recorded in this study is its potential as a contributor to the establishment, spread and persistence of non-native invasive plant species. The establishment and spread of these plants are an increasingly important issue for land managers and conservation of the native forests and shrublands in protected areas and national parks. In the case of *C. vulgare* (exotic forb species), there is evidence that the alteration of habitat by fire, herbivory or cutting favors its establishment and colonization, resulting in more visits by pollinators in disturbed environments (Morales 2005). Our results showed that cattle did not decrease the amount of fruits, *C. vulgare* being the most abundant at least during early regeneration (Table 1).

The effects of livestock reported in this study are, therefore, relevant to the understanding of how browsing can alter native species regeneration and possibly change successional trajectories following the burning of *Nothofagus* forests. As this study of browsing effects on reproduction phenology shows strong contrasting patterns between palatable and non-palatable species, further research is needed on the potential effects on pollinator guilds and disperser species.

FUNDING

ACKNOWLEDGEMENTS

REFERENCES

Abdala-Roberts L, Parra-Tabla V, Navarro J (2007) Is floral longevity influenced by reproductive costs and pollination success in Cohniella ascendens (Orchidaceae)? *Ann Bot* **100**:1367–71.

Aizen MA (2007) Enfoques en el estudio de la reproducción sexual de las plantas en ambientes alterados: limitaciones y perspectivas. *Ecologia Austral* **17**:7–19.

Aizen MA, Feinsinger P (1994) Forest fragmentation, pollination, and plant reproduction in a Chaco Dry Forest, Argentina. *Ecology* **75**:330–51.

Arroyo MTK, Armesto JJ, Villagran C (1981) Plant phenological patterns in the High Andean Cordillera of Central Chile. *J Ecol* **69**:205–23.

Aximoff IA, Freitas L (2010) Is pollen removal or seed set favoured by flower longevity in a hummingbird-pollinated Salvia species? *Ann Bot* **106**:413–19.

Billings WD (1990) Bromus tectorum, a biotic cause of ecosystem impoverishment in the Great Basin. In Woodwell, G. M. (ed). *The Earth in Transition: Patterns and Processes of Biotic Impoverishment*. New York: Cambridge University Press, 301–22.

Blackhall M (2006) *Efecto del ganado sobre la regeneración temprana post-fuego de un bosque mixto de Nothofagus dombeyi y Austrocedrus chilensis*. San Carlos de Bariloche, Argentina: Universidad Nacional del Comahue.

Blackhall M, Raffaele E, Veblen TT (2008) Cattle affect early post-fire regeneration in a Nothofagus dombeyi-Austrocedrus chilensis mixed forest in northern Patagonia, Argentina. *Biol Conserv* **141**:2251–61.

Blair AC, Wolfe LM (2007) The association between floral longevity and pollen removal, pollen receipt, and fruit production in flame azalea (Rhododendron calendulaceum). *Can J Bot* **85**:414–19.

Brown VK, Gange AC, Evans IM, *et al.* (1987) The effect of insect herbivory on the growth and reproduction of two annual Vicia species at different stages in plant succession. *J Ecol* **75**:1173–89.

Brooks ML, D'Antonio CM, Richardson DM, *et al.* (2004) Effects of invasive alien plants on fire regimes. *Bioscience* **54**:677–88.

Cavallero L. (2012) Heterogeneidad ambiental y dispersión de semillas en comunidades de distinta edad post-fuego del noroeste de Patagonia (Tesis Doctoral)., S.C. de Bariloche, Argentina: Universidad Nacional del Comahue, *Centro Regional Universitario Bariloche*.

Conover WJ (1980) *Practical Nonparametric Statistics*. New York: John Wiley & Sons.

Correa MN (1976) Flora Patagónica. Instituto Nacional de Tecnología Agropecuaria, Buenos Aires, Argentina.

D'Antonio CM, Tunison JT (2000) Variation in impact of exotic grass fueled fires on species composition across an elevation gradient in Hawai'i. *Austral Ecol* **25**:507–22.

Damascos MA, Rapoport EH (2002) Diferencias en la flora herbácea y arbustiva entre claros y áreas bajo dosel en un bosque de Nothofagus pumilio en Argentina. *Revista Chilena de Historia Natural* **75**:465–72.

Dormann CF, Bakker JP (2000) The impact of herbivory and competition on flowering and survival during saltmarsh succession. *Plant Biol* **2**:68–76.

Elton CS (1958) *The Ecology of Invasions by Animals and Plants*. London, UK: Methuen.

Forcella F, Webster T, Cardina J (2004) Protocolos para la determinación de bancos de semillas de malezas en los agrosistemas. In Labrada R (ed). *Manejo de malezas para países en desarrollo. Addendum 1*. Roma: FAO, 3–21.

Ghermandi L, Guthmann N, Bran D, *et al.* (2004) Early post-fire succession in northwestern Patagonia grasslands. *J Vegetat Sci* **15**:67–76.

Gómez JM (1996) Predispersal reproductive ecology of an arid land crucifer, Moricandia moricandioides: effect of mammal herbivory on seed production. *J Arid Environ* **33**:425–37.

Gómez JM, González-Megías A (2007) Long-term effects of ungulates on phytophagous insects. *Ecol Entomol* **32**:229–34.

Henrich B (1976) Flowering phenologies: bog, woodland and disturbed habitats. *Ecology* **57**:890–99.

Herrera J (1986) Flowering and fruiting phenology in the coastal shrublands of Doñana, south Spain. *Plant Ecol* **68**:91–98.

Ivey CT, Carr DE (2005) Effects of herbivory and inbreeding on the pollinators and mating system of *Mimulus guttatus* (Phrymaceae). *Am J Bot* **92**:1641–9.

Juenger T, Bergelson J (1998) Pairwise versus diffuse natural selection and the multiple herbivores of Scarlet Gilia, Ipomopsis aggregata. *Evolution* **52**:1583–92.

Kitzberger T, Raffaele E, Heinemann K, *et al.* (2005) Effects of fire severity in a north Patagonian subalpine forest. *J Vegetat Sci* **16**:5–12.

Kitzberger T, Veblen TT (1999) Fire-induced changes in northern Patagonian landscapes. *Landsc Ecol* **14**:1–15.

Lehtilä K, Strauss S (1999) Effect of foliar herbivory on male and female reproductive traits of wild radish, Raphanus raphanistrum. *Ecology* **80**:116–24.

Lehtilä K, Strauss SY (1997) Leaf damage by herbivores affects attractiveness to pollinators in wild radish, Raphanus raphanistrum. *Oecologia* **111**:396–403.

Menzel A, Sparks TH, Estrella N, *et al.* (2006) European phenological response to climate change matches the warming pattern. *Glob Change Biol* **12**:1969–76.

Morales CL (2005) *Alteración del hábitat e interacciones entre especies nativas y exóticas a través de la polinización de los bosques templados de Sudamérica austral*. San Carlos de Bariloche, Argentina: Universidad Nacional del Comahue.

Mothershead K, Marquis RJ (2000) Fitness impacts of herbivory through indirect effects on plant-pollinator interactions in Oenothera macrocarpa. *Ecology* **81**:30–40.

Nuñez M, Bailey J, Schweitzer J (2010) Population, community and ecosystem effects of exotic herbivores: a growing global concern. *Biol Invasions* **12**:297–301.

Nuñez MA, Raffaele E (2007) Afforestation causes changes in post fire regeneration in native shrubland communities of northwestern Patagonia, Argentina. *J Vegetat Sci* **18**:827–834.

Opler PA, Frankie GW, Baker HG (1980) Comparative phenological studies of treelet and scrub species in tropical wet and dry forest in the lowlands of Cost Rica. *J Ecol* **68**:167–88.

Paritsis J, Raffaele E, Veblen TT (2006) Vegetation disturbance by fire affects plant reproductive phenology in a srhubland community in northwestern Patagonia, Argentina. *New Zeal J Ecol* **30**:387–95.

Poveda K, Steffan-Dewenter I, Scheu S, *et al.* (2003) Effects of below- and above-ground herbivores on plant growth, flower visitation and seed set. *Oecologia* **135**:601–5.

Quinn GP, Keough MJ (2002) *Experimental Design and Data Analysis for Biologists*. Cambridge, UK: Cambridge University Press.

Raffaele E, Veblen TT (1998) Facilitation by nurse shrubs of resprouting behaviour in a post-fire shrubland in northern Patagonia, Argentina. *J Vegetat Sci* **9**:1–6.

Raffaele E, Veblen TT (2001) Effects of cattle grazing on early postfire regeneration of matorral in Northwest Patagonia, Argentina. *Nat Areas J* **21**:243–9.

Raffaele E, Veblen TT, Blackhall M, *et al.* (2011) Synergistic influences of introduced herbivores and fire on vegetation change in northern Patagonia, Argentina. *J Vegetat Sci* **22**:59–71.

Relva MA, Veblen TT (1998) Impacts of introduced large herbivores on Austrocedrus chilensis forests in northern Patagonia, Argentina. *For Ecol Manag* **108**:27–40.

Sasal Y (2009) *Efecto del ganado en áreas incendiadas sobre la comunidad de artrópodos terrestres y las interacciones Planta-insecto en el NO de la Patagonia, Argentina*. S. C. de Bariloche, Argentina: Universidad Nacional del Comahue, Centro Regional Universitario Bariloche.

Silvertown J, Smith B (1989) Germination and population structure of spear thistle Cirsium vulgare in relation to experimentally controlled sheep grazing. *Oecologia* **81**:369–73.

Smith-Ramírez C, Armesto JJ, Figueroa J (1998) Flowering, fruiting and seed germination in Chilean rain forest myrtacee: ecological and phylogenetic constraints. *Plant Ecol* **136**:119–31.

Sokal RR, Rohlf FI (1969) *Biometry: The Principles and Practice of Statistics in Biological Research*. San Francisco, CA: W.H. Freeman & Co.

Tercero-Bucardo N, Kitzberger T, Veblen TT, *et al.* (2007) A field experiment on climatic and herbivore impacts on post-fire tree regeneration in north-western Patagonia. *J Ecol* **95**:771–9.

Varela SA, Gobbi ME, Laos F (2006) Banco de semillas de un bosque quemado de Nothofagus pumilio: efecto de la aplicación de compost de biosólidos. *Ecología Austral* **16**:63–78.

Vázquez DP (2002) Multiple effects of introduced mammalian herbivores in a temperate forest. *Biol Invasions* **4**:175–91.

Vázquez DP, Simberloff D (2003) Changes in interaction biodiversity induced by an introduced ungulate. *Ecol Lett* **6**:1077–83.

Vázquez DP, Simberloff D (2004) Indirect effects of an introduced ungulate on pollination and plant reproduction. *Ecol Monogr* **74**:281–308.

Veblen T, Kitzberger T, Raffaele E, *et al.* (2003) Fire history and vegetation changes in northern Patagonia, Argentina. In Veblen TT, Baker WL, Montenegro G, *et al.* (eds). *Fire and Climatic Change in Temperate Ecosystems of the Western Americas*. New York: Springer, 265–95.

Veblen TT, Lorenz DC (1987) Post-fire stand development *of Austrocedrus-Nothofagus* forests in northern Patagonia. *Veget Hist Archaeobot* **71**:113–26.

Veblen TT, Martin C, Mermoz M, *et al.* (1989) Effects of exotic deer on forest structure and composition in northern Patagonia. *J Appl Ecol* **26**:711–24.

Veblen TT, Armesto JJ, Burns BR, *et al.* (2005) The coniferous of South America. In Andersson F, Gessel S (eds). *Ecosystems of the World: Coniferous Forests*. Amsterdam: Elsevier, 701–25.

Veblen TT, Holz A, Paritsis J, *et al.* (2011) Adapting to global environmental change in Patagonia: what role for disturbance ecology. *Austral Ecol* **36**:891–903.

Veblen TT, Mermoz M, Martin C, *et al.* (1992a) Ecological impacts of introduced animals in Nahuel Huapi National Park, Argentina. *Conserv Biol* **6**:71–83.

Veblen TT, Thomas K, Lara A (1992b) Disturbance and forest dynamics along a transect from Andean Rain Forest to Patagonian shrubland. *J Vegetat Sci* **3**:507–20.

Verboom GA, Stock WD, Linder HP (2002) Determinants of postfire flowering in the geophytic grass Ehrharta capensis. *Funct Ecol* **16**:705–13.

Zar JH (1996) *Biostatistical Analysis*. Englewood Cliffs, NJ: Prentice-Hall, Inc.