

# Mountain invasions on the way: are there climatic constraints for the expansion of alien woody species along an elevation gradient in Argentina?

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## Abstract

### Aims

Increasing evidence worldwide indicates that high mountain regions are not immune to invasion by alien plants. Here, we aimed to address whether three major woody invaders of low-mountain areas are constrained to lower altitudes due to climatic restrictions or just by low propagule pressure. We hypothesize that the increasing climatic harshness towards higher altitudes restricts seedling establishment and survival of these woody invaders and thus their potential for altitudinal expansion.

### Methods

The study was carried out in the Sierras Grandes Mountains, Córdoba, central Argentina (32°50'S, 64°90'W). We addressed the hypothesis with an experimental approach, dissociating the effect of the environmental gradient from that of propagule pressure, by discarding the influence of seed sources. We translocated seeds of *Gleditsia triacanthos* L., *Ligustrum lucidum* W.T. Aiton. and *Pyracantha angustifolia* (Franch.) C.K.Schneid. along the altitudinal gradient (from 900 to 2700 m asl). Seven sites were selected along the altitudinal gradient, spaced every 200–400 m. Three plots (4 × 4 m) were selected within each altitudinal site and excluded from livestock; 100 seeds of each species were sown within each plot (6300 seeds in total). Seedling emergence and survival was monitored during two growing seasons and soil temperature and moisture was recorded as well. The field experiment was complemented with lab assays.

### Important Findings

Climate along this altitudinal gradient might be least restrictive at mid elevations, as a result of the intersection between opposite soil temperature and moisture gradients. Sown seeds germinated along the whole altitudinal gradient and seedlings successfully established and overwintered well above their current altitudinal distribution (up to 2200 m for *Ligustrum* and 2400 m for *Gleditsia* and *Pyracantha*). Additional lab assays confirmed field patterns and gave some insights into contrasting regeneration strategies between these invaders that might help to overcome stochastic environmental constraints in the germination stage. Overall, seedlings of three major woody invaders of low-mountain woodlands can establish and survive at higher elevations than their current distribution. In contrast to natives, they seem to be broad climate tolerators, rather than specialized stress tolerators, capable of germinating and growing across a wide elevational range. While long-term experiments might be necessary to fully address the potentials for altitudinal expansion, our results on early lifecycle stage suggest that the invaders studied here would have mostly a dispersal barrier rather than a climate barrier to establish in the upper belt of the Sierras Grandes.

**Keywords:** altitudinal gradient, exotic species, *Gleditsia triacanthos*, *Ligustrum lucidum*, *Pyracantha angustifolia*, seedling establishment

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## INTRODUCTION

Biological invasions are recognized as a major driver of biodiversity decline and altered ecosystem services worldwide (Mack *et al.* 2000; Sala *et al.* 2000). Despite the traditional belief that mountains are less prone to invasions than other ecosystems, there is increasing attention to the expansion of alien species over high elevation environments (Dietz *et al.* 2006; Pauchard *et al.* 2009). In this regard, consistent patterns of declining non-native plant species richness with increasing elevation have been described in several mountainous areas (Alexander *et al.* 2011; Arévalo *et al.* 2005; Becker *et al.* 2005; McDougall *et al.* 2011; Pollnac and Rew 2014). Broad climatic tolerance combined with enough residence time in a given region seem to underlie the success of non-native species at high elevations ranges (Alexander *et al.* 2011; Haider *et al.* 2010; Pyšek *et al.* 2011).

Most of the evidence on the occurrence of alien flora along elevational gradients comes from species that are already established in their non-native ranges. However, current altitudinal limits of plant distribution may not necessarily reflect their actual climatic tolerance (Araújo and Pearson 2005). For instance, an invader may not occur in climatically suitable areas just because the time since introduction has not been enough to reach those altitudes (considering that propagule sources are concentrated in lower areas). It is difficult to isolate the incidence of climatic constraints in altitudinal gradients from other factors through observational studies on established species (Pauchard and Alaback 2004). In this context, experimental seed addition of alien herbs has proven to be a useful way forward to address climatic restrictions while controlling for propagule pressure (Petryna *et al.* 2002; Paiaro *et al.* 2007; Poll *et al.* 2009). This type of experimental approach might be particularly necessary to assess for climatic constraints at high elevations in which alien species are currently absent.

Despite their importance in invasion ecology (Richardson *et al.* 2014), expansion of woody aliens along altitudinal ranges has been poorly studied in comparison to other growth forms such as herbs (McDougall *et al.* 2011). In central Argentina, Córdoba Mountain range includes a wide environmental gradient (500–2790 m asl) that is subject to an incipient spread of woody alien species from lower altitudes (Giorgis *et al.* 2011a). At present, the alien species *Gleditsia triacanthos*, *Ligustrum lucidum* and *Pyracantha angustifolia* have been reported to be the most successful invaders of low-mountain ecosystems in the region (Giorgis *et al.* 2011b; Gavier-Pizarro *et al.* 2012; Hoyos *et al.* 2010; Marco and Paez 2000; Tecco 2006; Tecco *et al.* 2013). Although their dispersal agents are present throughout the gradient (i.e. domestic livestock for *Gleditsia* and native bird dispersers for *Ligustrum* and *Pyracantha*; Badini 2012; Ferreras *et al.* 2015; Miatello *et al.* 1999; Montaldo 2005; Marcora *et al.* 2013), only *Pyracantha* has already reached higher altitudes (up to 1700 m, albeit with low frequency), while *Ligustrum* and *Gleditsia* still remain

below 1500 m (Giorgis 2011; Giorgis *et al.* 2011a). While low propagule pressure might restrict their altitudinal spread (Giorgis *et al.* 2011a, Giorgis *et al.* 2011b), the relevance of climatic constraints has not still been explored as a further driver of their absence at higher elevations (Pauchard *et al.* 2009). We hypothesize that the increasing climatic harshness towards higher altitudes restricts seedling establishment and survival of the major woody invaders of low-mountain areas and thus their potential for altitudinal expansion. Alternatively, biotic constraints such as propagule pressure might be driving their current altitudinal limits.

## METHODS

### Study site

The study was conducted in the Sierras Grandes Mountains, Córdoba, central Argentina. The experimental plots were placed along an altitudinal gradient ranging from 900 m asl up to the highest altitude of the mountain range, at 2700 m (Linderos Road, 32° 50'S, 64° 90'W). The whole gradient comprises the following vegetation belts described by Cabrera (1976) for the Mountain Chaco District: (i) the upper portion of Chaco mountain woodlands, distributed between 400 and 1300 m; (ii) an intermediate belt devoid of forest currently occupied by mountain grasslands and shrublands (1300–1700 m; Giorgis, 2011) and (iii) a mosaic of high mountain grasslands and *Polylepis australis* woodlands (above 1700 m). Following the Köppen–Geiger climate classification, the region is subjected to a Temperate/Mesothermal climate (C), with dry winters (Cw). Along the altitudinal gradient, the climate varies from warm-temperate with dry season and warm summer (Cwa) in the lower parts of the mountain to temperate/cold temperate with dry season and mild summers (Cwb) in the upper belt. Specifically, mean annual temperature at the lower end of the gradient (900 m) is 15.7°C dropping to 7.4°C at the summit, at 2700 m (Marcora *et al.* 2008). There is no frost-free period above 1800 m. Mean annual precipitation vary between 750 and 970 mm, with most rainfall concentrated in the warmer months, from October to April (Cabido 1985; Colladon *et al.* 2010). The main economic activity is livestock rearing (sheep and cattle) that began in the early 17th century and had completely replaced the native herbivores (*Lama guanicoe*) by the beginning of the 20th century (Díaz *et al.* 1994).

### Study species

*Gleditsia triacanthos* L., Fabaceae, is a thorny tree native to North America, consumed and dispersed by cattle (Ferreras and Galetto 2010). *Ligustrum lucidum* W. T. Aiton, Oleaceae ('glossy privet'), is an evergreen Asiatic tree, with bird-dispersed fleshy fruits (Montaldo 1993). *Pyracantha angustifolia* (Franch.) C.K. Schneid., Rosaceae (fire hawthorn), is an evergreen, thorny Asiatic shrub with bird-dispersed fleshy fruits (Novara 1993). Mean seed weight of these species in Chaco mountain woodlands are 204.78 mg ± 12.03 for *Gleditsia*,

17.83 mg  $\pm$  0.293 for *Ligustrum* and 3.995 mg  $\pm$  0.1079 for *Pyracantha* (Tecco *et al.* 2013).

The three species were originally introduced as ornamentals and have been reported as aggressive invaders both in grasslands and woody ecosystems of Argentina (Chaneton *et al.* 2004; Delucchi 1991; Ferreras and Galetto 2010; Gavier-Pizarro *et al.* 2012; Ghersa *et al.* 2002; Giorgis 2011; Hoyos *et al.* 2010; Lichstein *et al.* 2004; Marco and Páez 2000; Mazia *et al.* 2001, 2010; Tecco *et al.* 2006; Zeballos *et al.* 2014a) and other parts of the world (Rejmánek and Richardson 2013).

### Experimental design

We conducted an experimental approach to test whether seedling success of the invasive species were restricted by the environmental gradients that characterize these mountains (Table 1). Site selection attempt to minimize biotic differences between altitudes and seed addition allowed dissociating the effect of abiotic constraints from those of propagule pressure. The field experiment was preceded and complemented with lab assays evaluating potential germination rates under manipulated temperature regimes (see below). We used seed germination and seedling growth and survival across the gradient as proxy of species' potential for altitudinal expansion, considering that this stage of the lifecycle is decisive for the success of the invasion (Rejmánek 2000, but see Flores-Moreno and Moles 2013) as well as a stage strongly affected by climatic constraints (Carrillo-Gavilán *et al.* 2012).

Seven altitudinal sites were selected along the gradient, placed at intervals of about 200–400 m (963, 1243, 1600, 1803, 2248, 2458 and 2685 m). All sites were short grasslands, established on ridges with similar gentle slopes and high solar insolation (see Marcora *et al.* 2013 for more details). Livestock enclosures were built and three plots (4  $\times$  4 m) were selected inside, at each altitudinal site. For simplicity, altitudinal sites will be hereafter named by elevation: 900, 1200, 1600, 1800, 2200, 2400 and 2700 m.

We characterized the climatic gradient by measuring the annual and seasonal variation in soil temperature and moisture in all plots from April 2008 to April 2011. Soil moisture was measured monthly at 5 cm depth with a 'Moisture Probe Meter' (MPB-160-B del ICT International Pty Ltd). Soil temperature was monitored every 6 h (7:00, 13:00, 19:00 and 1:00 h) with iButtons buried at 5 cm depth in each plot. Some iButtons were damaged or lost during some intervals of the experiment, thus having an unbalanced number of records in each replicate. Month mean, maximum and minimum temperatures across the gradient were calculated for each plot pooling the 3 years of records. Soil of each altitudinal site was also characterized in terms of texture, pH, organic matter, carbon and phosphorus and nitrogen content. Soil properties were measured on three compound samples per altitude, from the top 15 cm (7 altitudes  $\times$  3 plots = 21 compound soil cores). Soil physiochemical properties are provided as a general site descriptor based on one collection in spring following the end of the present experiment (September 2011).

### Fruit collection and seed pooling

Fruits were collected from at least 50 individuals of each species, randomly selected across the whole range of distribution. According to species phenology, fruit collection was concentrated on autumn–winter 2009. The fleshy mesocarp of *Pyracantha* and *Ligustrum* fruits was removed and seeds of *Gleditsia* were individually scarified to overcome the physical dormancy. Seeds of each species were pooled, in order to standardize any differences associated with local seed origin as well as incorporating genetic variability. The same pool of seeds was used for lab and field experiments.

### Seedling emergence, survival and growth in the field

The field experiment was carried out between the end of August 2009 and mid-April 2011. The first date was chosen to coincide with the last period of the dry and cold season (i.e. just before the first rains). The end of the experiment was chosen to coincide with the end of the second growing season of seedlings. To measure seedling emergence, 100 seeds of each species were placed on the soil surface within each plot (August 26th 2009). Trying to simulate the natural process as much as possible, seeds were not buried, but they became naturally covered by litter and neighboring vegetation after being sown. In order to minimize soil-related and biotic differences between altitudes, the micro sites chosen for seed addition were in all cases similar patches of short grasses and herbs with no bare soil. That is, seeds were never placed under tall tussocks or shrubs to preclude any local moderating of climatic constraints (Cavieres *et al.* 2006). A total of 6300 seeds were planted in the study site (3 species  $\times$  100 seeds  $\times$  3 plot replicates  $\times$  7 altitude treatments). Seeds were protected from seed predation with cylindrical cages staked to the ground. Rings of 45 cm diameter  $\times$  25 cm height were built with a 2-cm mesh stainless solid net and walls of these cylindrical structures were further covered with a 1-mm mesh stainless net. The upper extreme of the rings were also covered with a 1-cm mesh stainless net. No net was placed at the bottom of the rings. Cages did not prevent arthropod herbivory or neighboring vegetation growth.

Seeds were monitored monthly. Seedling emergence started 3–4 months after sowing (see Results). Survival percentage of these seedlings was monitored every 2 months during the next 1.5 year. Seedlings were harvested in autumn, at the end of their second growing season (April 15th 2011), in order to record growth performance. The growth variables considered were: plant height, stem diameter at the base (DAB), total above-ground biomass, leaf biomass and number of leaves and buds.

Special efforts were made to remove any propagules or ramets left in the study site after harvesting the seedlings to avoid experimental introductions of invasive species.

### Lab germination experiments

Potential germination of the three species was characterized prior to the field experiment simulating the range of soil

**Table 1:** environmental characterization of sites along an altitudinal gradient in the Sierras Grandes Mountains, Córdoba, central Argentina

Variable	Altitude										Adj $R^2$	P
	900	1200	1600	1800	2200	2400	2700					
Soil temperature (°C)												
Mean	15.45 ± 0.44	13.01 ± 0.32	13.03 ± 1.18	10.79 ± 1.61	10.87 ± 1.05	7.93 ± 0.29	6.38 ± 0.48	0.94	0.0002			
Maximum	18.81 ± 0.61	15.16 ± 0.52	16.61 ± 0.58	13.21 ± 1.64	12.73 ± 1.13	9.83 ± 0.8	7.82 ± 0.48	0.91	0.0005			
Minimum	12.84 ± 0.3	11.45 ± 0.15	10.47 ± 1.38	9.02 ± 1.6	9.24 ± 1	6.45 ± 0.22	5.26 ± 0.52	0.95	0.0001			
Soil moisture (%)												
Mean	22.35 ± 0.3	22.83 ± 2.08	21.63 ± 1.71	34.81 ± 2.85	43.63 ± 2.84	44.98 ± 1.83	50.02 ± 2.63	0.88	0.0011			
Maximum	26.47 ± 0.44	27.06 ± 2.35	25.72 ± 1.58	41.48 ± 3.24	50.73 ± 3.49	53.33 ± 2.15	57.94 ± 2.31	0.88	0.001			
Minimum	17.85 ± 0.18	18.33 ± 1.97	17.28 ± 1.73	28.08 ± 2.7	36.70 ± 2.19	37.20 ± 1.91	42.81 ± 2.67	0.88	0.0011			
Soil texture												
Sand	72.57 ± 1.24	74.63 ± 2.97	77.00 ± 2.79	76.73 ± 3.74	51.00 ± 1.28	74.23 ± 2.02	57.70 ± 5.84	0.14	ns			
Slime	16.53 ± 0.5	14.27 ± 2.17	16.43 ± 1.6	17.70 ± 3.22	38.90 ± 0.85	17.20 ± 2.71	30.87 ± 5.63	0.21	ns			
Clay	10.90 ± 0.76	11.10 ± 0.8	6.57 ± 1.19	5.57 ± 0.52	10.10 ± 0.49	8.57 ± 1.86	11.43 ± 0.29	0	ns			
Soil chemical prop.												
Org. matter (%)	4.23 ± 0.26	5.73 ± 0.34	5.20 ± 1.06	9.47 ± 1.07	9.67 ± 0.41	16.23 ± 1.83	13.07 ± 0.07	0.79	0.0048			
Org. carbon (%)	2.43 ± 0.15	3.33 ± 0.19	3.03 ± 0.62	5.50 ± 0.64	5.60 ± 0.27	9.40 ± 1.04	7.60 ± 0	0.79	0.0046			
Nitrogen (%)	0.20 ± 0.01	0.27 ± 0.02	0.25 ± 0.04	0.45 ± 0.05	0.45 ± 0.02	0.79 ± 0.1	0.61 ± 0.01	0.76	0.0069			
C:N	12.17 ± 0.2	12.27 ± 0.15	12.00 ± 0.5	12.10 ± 0.21	12.30 ± 0.15	12.03 ± 0.26	12.40 ± 0.06	0	ns			
Phosphorus (ppm)	4.27 ± 3.32	10.80 ± 3.41	2.07 ± 0.38	6.93 ± 0.97	2.00 ± 0.12	0.77 ± 0.38	1.37 ± 0.39	0.25	ns			
pH	5.80 ± 0.25	5.77 ± 0.29	4.83 ± 0.07	4.23 ± 0.15	4.73 ± 0.03	5.13 ± 0.03	5.07 ± 0.22	0.09	ns			

Annual means of soil temperature and moisture were obtained from 3-year records (autumn 2008–autumn 2011;  $n = 3$  per altitude). Physical and chemical soil properties correspond to a single record (spring 2011;  $n = 3$  per altitude). Altitudinal means ± SE and linear regression statistics (adjusted  $R^2$  and  $P$ ) are provided.

temperatures present in the growing season across the altitudinal gradient. The coldest treatment emulated the range of mean minimum and maximum soil temperatures present at the highest altitude (5.99 and 9.19°C, respectively; measured from November to March in 2008–2010; Pais-Bosch et al. 2012). Intermediate temperature regimes as well as an extreme warm treatment were also considered in order to cover a broad (though possible) spectrum of conditions in the study region (Nuñez et al. 2008).

For each species, 30 seeds were placed in 9 cm diameter Petri dishes on filter paper moistened with distilled water. Seeds were incubated in a chamber under controlled day and night light conditions (12/12 h daily photoperiod of ~38 mmol/m<sup>2</sup>/s, provided by cool white fluorescent light tubes). A darkness treatment was also tested by wrapping half of the dishes with a layer of aluminum foil. Five different temperature regimes of 10/5°C, 15/5°C, 20/10°C, 25/15°C and 35/20°C were used, alternating each temperature every 12 h. A total of 1500 seeds were incubated per species (5 temperature treatments × 2 light treatments × 5 replicates × 30 seeds). Germination in day/night light conditions was monitored daily and filter paper was moistened with distilled water when necessary until the end of the experiment. Germination at continuous darkness was recorded at the end of the corresponding essays. Protrusion of the radicle was the criterion for germination (Tecco et al. 2012).

### Data analysis

We assessed the variation along the altitudinal gradient of soil temperature, moisture and physicochemical properties using linear regressions. It has been suggested that for altitudinal environmental gradient analysis to become biologically meaningful, the temperatures that need to be considered are those that become physiologically effective—that is, those occurring during the growing season (*sensu* Körner 2007). For this reason, the general environmental characterization was complemented with an analysis of the seasonal variation in soil temperature and moisture along the altitudinal gradient. For this, we ran general linear model (GLM) with a Gaussian error structure correcting for the heteroscedasticity in some variables, using the function `varIdent` in `Infostat` (Di Rienzo et al. 2014) and its interface to R (R 2.15.1, R development core team 2012). A first model with altitude, season and their interaction as fixed factors was run. Four independent models were then run to assess differences among altitudes within each season.

Response variables in the field experiment were: (i) maximum seedling emergence (highest % of seedlings present after seed planting), (ii) seedling growth (means for all growth variables after two growing seasons) and (iii) seedling survival (% seedlings present at the end of the second growing season). This percentage was evaluated on the subset of seedlings alive at the end of the first growing season ( $T_0$  = March 2010,  $T_1$  = April 2011). Differences in terms of maximum seedling emergence between altitudes were assessed using GLM with a binomial distribution (R 2.15.1, R development core

team 2012). The moderate seedling emergence plus the high mortality at the end of the first growing season resulted in quite low  $n$  values for the three species across the gradient (i.e. 0–3 cages per altitude with 1–35 saplings within cages, see Supplementary Table 1 for total number of harvested saplings). Unfortunately, this precluded a formal statistical analysis on survival and growth data. Means are nevertheless shown given their relevant biological meaning.

The percentage of germinated seeds across the different temperature regimes were analyzed with ANOVA and Fisher's *a posteriori* test for all species. The fixed factors used for these analyses were temperature, light and their interaction (R 2.15.1, R development core team 2012). In all the analysis, differences were considered significant at  $P < 0.05$ .

## RESULTS

### Environmental shifts along the altitudinal gradient

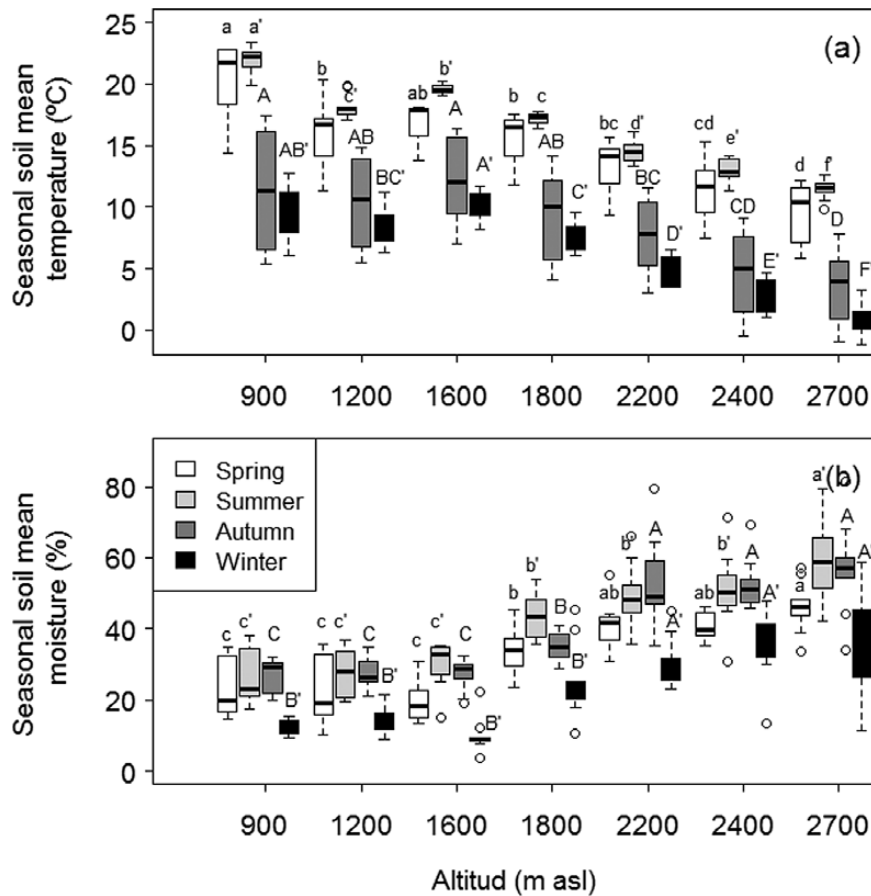
Consistent environmental changes were recorded along the altitudinal gradient for soil temperature, moisture and chemical properties. A significant decrease in soil temperature and an increase in soil moisture, nitrogen content, organic matter and carbon characterized the upper portions of the gradient (Table 1).

The environmental gradient in soil temperature and moisture was also consistent across seasons (Fig. 1). Mean soil temperature differed significantly between altitudes and seasons, with a non-significant interaction ( $F_{\text{altitude}} = 63.65$ ,  $P < 0.0001$ ;  $F_{\text{season}} = 572.97$ ,  $P < 0.0001$ ;  $F_{\text{altitude} \times \text{season}} = 1.12$ ,  $P = 0.3371$ ). Significantly decreasing temperatures were observed across the four seasons (summer, spring, autumn and winter, respectively; least significant difference (LSD) Fisher  $P < 0.05$ ) and main changes along the altitudinal gradient were observed above 1800 m, with significantly decreasing temperatures along each altitudinal site of the upper belt (2200, 2400 and 2700, respectively; LSD Fisher  $P < 0.05$ ). This general pattern was particularly evident in winter and summer, while more gradual in spring and autumn (Fig. 1a).

Mean soil moisture also differed significantly between altitudes and seasons, with a non-significant interaction ( $F_{\text{altitude}} = 78.00$ ,  $P < 0.0001$ ;  $F_{\text{season}} = 59.82$ ,  $P < 0.0001$ ;  $F_{\text{altitude} \times \text{season}} = 0.88$ ,  $P = 0.6012$ ). Soil moisture was significantly higher in summer and autumn, intermediate in spring and lowest in winter (LSD Fisher  $P < 0.05$ ). Main changes along the altitudinal gradient were observed between lower and dryer altitudes (900–1600 m) and the higher altitudes, which showed significant increasing soil moistures towards the upper portion of the gradient (1800, 2200, 2400 and 2700, respectively; LSD Fisher  $P < 0.05$ ). The overall moister soil conditions of the upper altitudinal sites were consistent through seasons (Fig. 1b).

### Seedling emergence, survival and growth in the field

Net seedling emergence was recorded across the whole altitudinal gradient for the three species. Seedling emergence



**Figure 1:** soil mean temperature (a) and moisture (b) across seasons along the altitudinal gradient in the Sierras Grandes Mountains (central Argentina). Annual means correspond to 3 years of records (2008–2011). Statistics of the general GLM model with altitude, season and their interaction as fixed factors are provided in the text. The additional four independent models are detailed here. For temperature by elevation, statistics were: Spring ( $F = 12.37$ ;  $P < 0.0001$ ), summer ( $F = 123.7$ ;  $P < 0.0001$ ), autumn ( $F = 7.37$ ;  $P < 0.0001$ ) and winter ( $F = 39.15$ ;  $P < 0.0001$ ). For soil moisture by elevation, statistics were: Spring ( $F = 19.90$ ;  $P < 0.0001$ ), summer ( $F = 20.17$ ;  $P < 0.0001$ ), autumn ( $F = 25.94$ ;  $P < 0.0001$ ) and winter ( $F = 20.09$ ;  $P < 0.0001$ ). Letters indicate mean differences between altitudes within each season (LSD Fisher,  $P < 0.05$ ). Differences within a single altitude are not indicated.

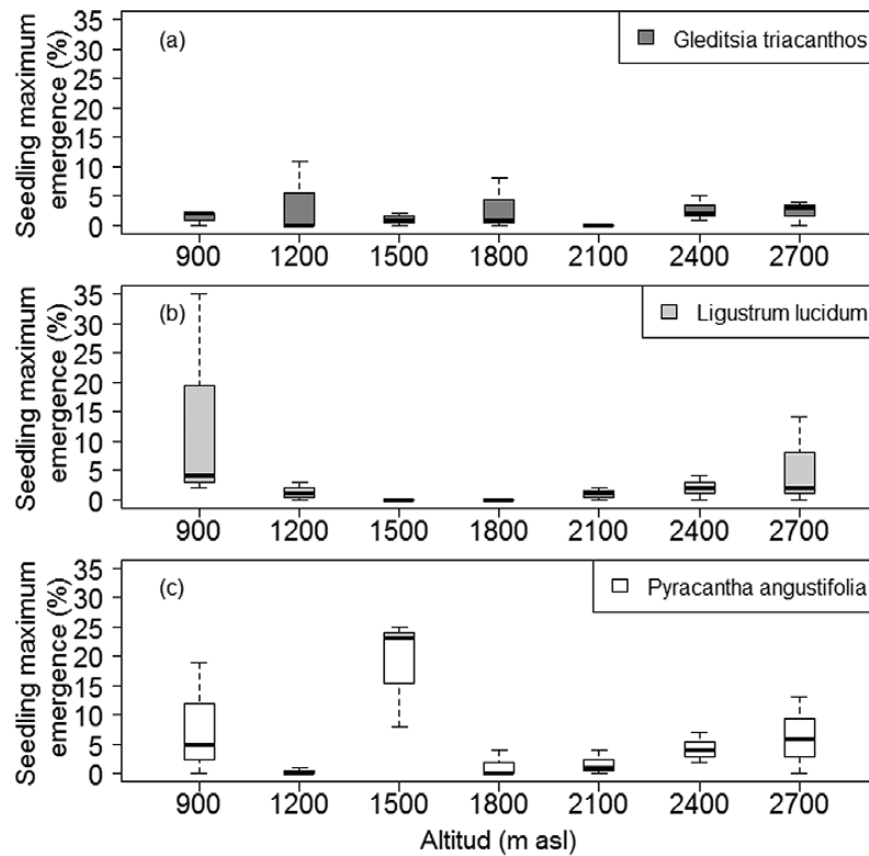
started 3–4 months after sowing, coinciding with the warmer and moister conditions prevailing in spring (2009). Seedlings of *Gleditsia* and *Pyracantha* were recorded from early November while those of *Ligustrum* started to emerge by mid-December. Soil mean temperature for both months ranged from 22°C at the lowest altitude to 11°C at the highest. Means of net emergence are summarized in Fig. 2. For *Gleditsia*, the peak of seedling emergence was achieved in spring close to the beginning of germination whereas *Ligustrum* and *Pyracantha* showed their maximum at the beginning of autumn. Germinated seeds of *Gleditsia* were found in the three plots at the 2200 m site, though radicles were desiccated. An activity accounted for the complete absence of *Ligustrum* seedlings at 1600 and 1800 sites.

The low number of *Gleditsia* seedlings that passed the first autumn showed surprisingly high percentages of survival during the winter (Fig. 3a). Healthy seedlings were harvested at 1600 and 2400 m at the end of the second growing season. Similarly, *Ligustrum* seedlings over-wintered throughout the gradient, but showed a progressive mortality at higher

altitudes in the following spring and summer (Fig. 3b). At the end of the second growing season, the highest altitude with seedlings still alive was 2200 m. Seedling survival was relatively constant at the first four altitudes (900–1800 m) for *Pyracantha*, while peaks of mortality were recorded at higher altitudes in the cold and following warm seasons (Fig. 3c). As for *Gleditsia*, the highest altitude where *Pyracantha* seedlings survived until harvesting, was 2400 m. Variation in seedling growth along the gradient could not be assessed statistically given the low final sample size (Supplementary Table 1). Seemingly, the highest values of plant height, stem diameter and biomass were recorded at the low to intermediate portions of the gradient (1600 m for *Gleditsia*, and 1200 for *Ligustrum* and *Pyracantha*).

### Potential germination across contrasting laboratory temperature regimes

The three species showed significant germination responses to the contrasting temperature regimes emulated in the lab



**Figure 2:** maximum seedling emergence recorded for the three woody alien species along an altitudinal gradient in the Sierras Grandes Mountains (central Argentina). Differences between altitudes were not significant in all cases (GLMs with binomial distribution). (a) *Gleditsia*,  $Z_{\text{altitude}} = 0$ ,  $P = 1$ ; (b) *Ligustrum*,  $Z_{\text{altitude}} = -0.40$ ,  $P = 0.68$  and (c) *Pyracantha*,  $Z_{\text{altitude}} = -0.195$ ,  $P = 0.84$ .

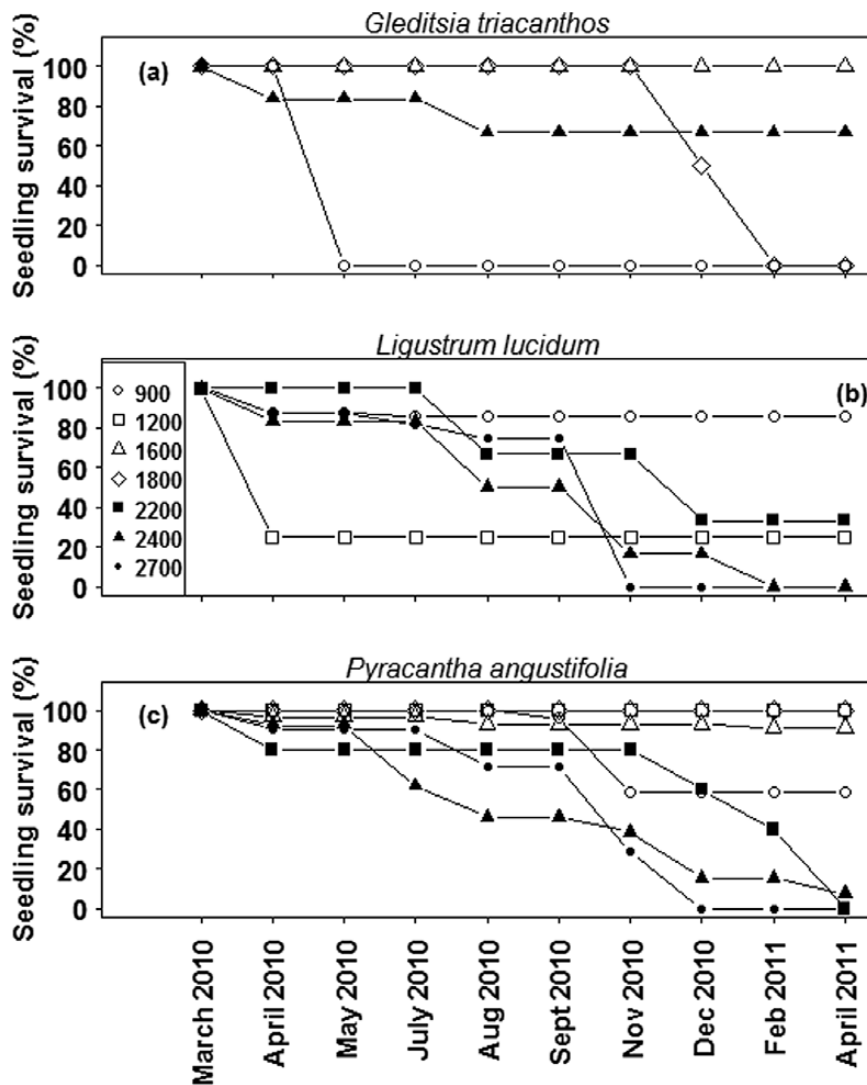
experiments (Fig. 4). *Gleditsia* had quite high germination potential in all conditions except for the coldest treatment where germination was negligible (Fig. 4a). Indeed, radicles of the very few seeds that germinated at 10/5°C stopped their growth soon after emergence ( $\leq 3$ mm). *Ligustrum* and *Pyracantha* also showed susceptibility to extreme temperature regimes (Fig. 4b and c). Neither of them was able to germinate at the coldest treatment (10/5°C) but had more than 60% germination at the temperate treatment (15/5°C) which is the most representative of the actual field temperatures in the growing season in the upper belt of the gradient. Unlike the other species, *Ligustrum* showed a significant decrease in germination potential with increasing temperatures, being almost null at the warmest treatment (35/20°C). While permanent darkness reduced germination percentages for *Gleditsia* and *Pyracantha*, means were biologically irrelevant (i.e. means for light/darkness versus permanent darkness were 80.60 versus 74.47% for *Gleditsia* and 59.80 versus 51.04% for *Pyracantha*, respectively).

Timing for germination was not equal for the three species (Fig. 5). *Gleditsia* seeds started to germinate following 7 days of incubation, and all seeds germinated simultaneously in a period of 2 days. It took 15 days for *Pyracantha* seeds to start

germinating, and all seeds germinated gradually in the subsequent 15 days. In the case of *Ligustrum*, it took 23 days to get the first germination record and germination progressed constant but slowly (i.e. 3–10% seeds per day) during more than 3 month (100 days).

## DISCUSSION

Increasing evidence worldwide indicates that high mountain regions are not immune to invasion by alien plants, though the spread of these species often occurs more slowly than in lowland areas (Dietz 2005; McDougall et al. 2011). In this study, we addressed whether the increasing climatic harshness towards higher altitudes restricts seedling establishment and survival of the three major woody invaders of low-mountain woodlands in central Argentina. Sown seeds germinated and seedlings successfully established and overwintered wide above their current altitudinal distribution (i.e. 1500–1700 m asl, see Introduction). This outcome contradicts our hypothesis and suggests that this species would have mostly a dispersal barrier rather than a climatic restriction to recruit in the upper mountain belt. Lab assays lend further support to these findings and give some insights into contrasting regeneration



**Figure 3:** seedling survival along an altitudinal gradient in the Sierras Grandes Mountains (central Argentina) for (a) *Gleditsia*, (b) *Ligustrum* and (c) *Pyracantha*. While seed addition was done on August 2009, percentages correspond to the subset of seedlings alive at the end of the first growing season ( $T_0$  = March 2010,  $T_1$  = April 2011).

strategies among these invaders that might help to overcome environmental stress in the germination stage (see below).

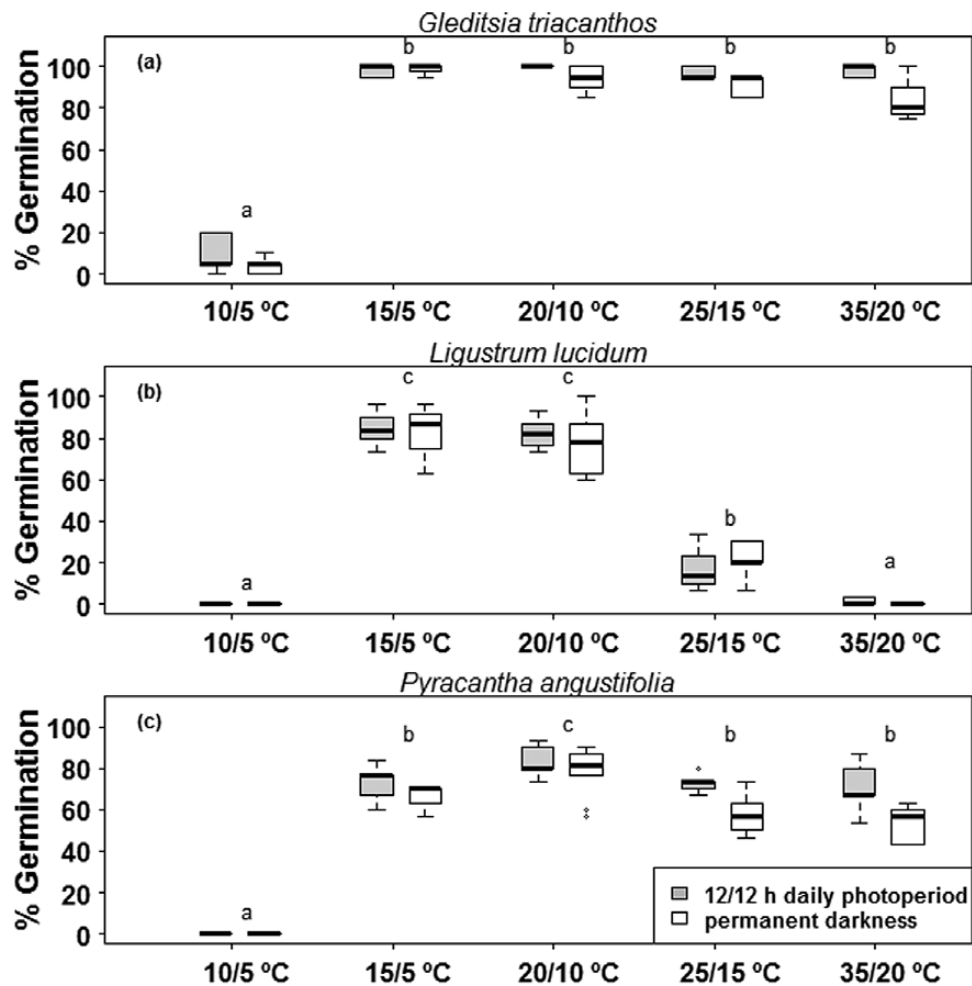
Studies across altitudinal gradients assume strong variation of climatic conditions which must be well documented to account for regional peculiarities, such as moisture availability (one of the most difficult and confusing components of altitudinal environmental gradients, *sensu* Körner 2007) and allow comparison with other mountains. Our data on soil temperature and moisture suggest that the climatic stress along this altitudinal gradient might not be unidirectional, but rather a combination of opposite temperature and moisture gradients. It seems that in mountains of this region, subjected to a dry winter, mid elevation might resume the most favorable condition for invasion (i.e. less extreme temperatures and more water availability in the dry season). This pattern with opposite direction of abiotic stressors has

also been described in other xeric mountains (Cavieres *et al.* 2006).

### Seedling emergence, survival and growth in the field

In general, extreme minimum temperature is recognized as an important climate factor constraining woody plant establishment and growth (Barni *et al.* 2012). This seemed to be the case for the three species analyzed in this study, especially in winter within the upper altitudinal belts (i.e. 2400 and 2700 m asl). Seedlings of the three species also appeared to have severe restrictions during the growing season since mortality peaked from September to April (i.e. spring to autumn; Fig. 5). Interactions such as competition with neighboring vegetation and herbivory by arthropods might have accounted for this high mortality of seedlings in spring-summer, though





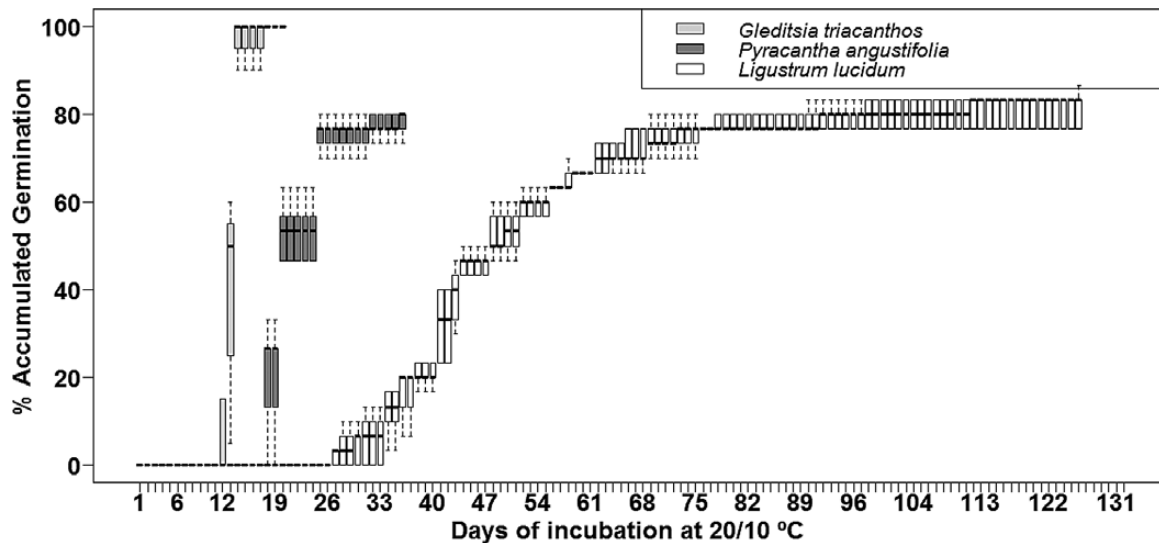
**Figure 4:** percentages of germination of (a) *Gleditsia*, (b) *Ligustrum* and (c) *Pyracantha* in five different temperature regimes alternating each temperature every 12 h (day/night). White bars correspond to 12/12 h daily photoperiod whereas grey bars correspond to permanent darkness. Letters indicate mean differences (LSD Fischer) between temperature treatments for *Gleditsia* ( $F_{\text{temp}} = 474.56$ ,  $P < 0.001$ ;  $F_{\text{light}} = 12.96$ ,  $P = 0.001$ ;  $F_{\text{temp} \times \text{light}} = 1.28$ ,  $P = 0.29$ ), *Ligustrum* ( $F_{\text{temp}} = 268.58$ ,  $P < 0.001$ ;  $F_{\text{light}} = 0.62$ ,  $P = 0.43$ ;  $F_{\text{temp} \times \text{light}} = 0.54$ ,  $P = 0.7$ ) and *Pyracantha* ( $F_{\text{temp}} = 150.47$ ,  $P < 0.001$ ;  $F_{\text{light}} = 12.48$ ,  $P = 0.001$ ;  $F_{\text{temp} \times \text{light}} = 1.79$ ,  $P = 0.14$ ).

experiments should be designed to confirm this hypothesis. Indeed, though climatic trends constitute the main drivers along altitudinal gradients from an ecological point of view (Körner 2007), we cannot rule out any further influence of soil quality (Table 1) and/or the interactions with soil biota along the altitudinal gradient (e.g. Defosse et al. 2011).

Our results show that the percentages of net seedling emergence were below 25%. These seemingly low values for the studied invasive species are similar to those reported in the same experimental setup for native woody species (Pais-Bosch et al. 2012), including the dominant tree of the upper altitudinal belts (*Polylepis australis*) and the dominant tree of low mountain woodlands (*Lithraea molleoides*). Unfortunately, data on seedling overwintering for these native species is not available to allow for further comparisons.

The absolute proportion of sown seeds that survived as seedlings for two growing seasons at the highest altitudes seems also rather low (i.e. 0.33% for *Ligustrum* at 2100 m,

1.33 and 0.33% for *Gleditsia* and *Pyracantha* at 2400, respectively). This is in accordance with many studies on seedling success at the treeline and below, which conclude that the first year poses the greatest risk, with not more than 1–3% of all germinates surviving the first winter (Körner 2012 and references therein). Indeed, our results are within the range of values reported in a comprehensive long term study (Renison et al. 2015) for the dominant native tree—*Polylepis australis*—in high mountain elevations in central Argentina (2260–2305 m). That is, seedling establishment of *P. australis* (percent of sown seeds that survived as seedlings after 5 years) ranged from 0.05 to 0.5% across contrasting topographies, at similar altitudes to those selected in this study (Renison et al. 2015). Moreover, our findings are in line with recent findings in which alien and native species do not significantly differ in germination, early seedling survival or even survival from germination to first reproduction (Flores-Moreno and Moles 2013). Low survival values might not hinder invasion



**Figure 5:** timing of germination of *Gleditsia* (gray), *Pyracantha* (dark gray) and *Ligustrum* (white). Means of accumulated germination percentages are provided for seeds incubated in a chamber under light/darkness (12/12 h) with a temperature regime of 20/10°C (alternating each temperature every 12 h; i.e. day/night). Means are provided until the last new germination occurred.

as shown for the invasive grass *Ampelodesmos mauritanica* in Mediterranean shrublands whose survival after 2 years was below 0.1% (Vila and Lloret 2000). In the case of the species studied here, surpassing the first growing season seems to be a strong bottleneck for a successful recruitment. Seedling survival between the end of the first growing season and the end of the second attained far higher values (i.e. 33.33% for *Ligustrum* at 2100 m, and 66.67% and 7.69% for *Gleditsia* and *Pyracantha* at 2400 m; Fig. 3) than those reported in another study (Vila and Lloret 2000).

Altogether, the results reported here suggest that the invasive woody species studied might not have higher seedling survival rates compared to each native at their optimal elevation but, instead, they are able to thrive well across most of the gradient except for the highest elevations suggesting a broader climatic tolerance (Alexander *et al.* 2011). Although restricted to the seedling stage, this broad climatic tolerance that seems to characterize the regeneration requirements of the three invaders gives support to the relevance of propagule pressure over climatic restrictions as the main constraint of altitudinal spread of woody invaders in these mountains.

### Potential germination across contrasting temperature regimes

Despite inability of seeds of the three invasive species to germinate at some extreme temperature treatments, all of them germinated at temperature ranges comparable to those that occur in the growing season along the whole altitudinal range. These findings are in line with field patterns and give further evidence against climatic restrictions at the germination stage in these mountains.

Different germination strategies seem to be at play among these invasive species to overcome stochastic environmental

constraints. These strategies range from long seed longevity that allows reliance upon a seed bank, to short seed longevity compensated by a long germination time. Both strategies allow, in a different way, a progressive input of new seedlings throughout the growing season. *Gleditsia* would belong to the first group (Ferrerías and Galetto 2010), *Ligustrum* to the second one, while *Pyracantha* seems to be in an intermediate situation. In the case of *Ligustrum*, the low seed longevity (Long *et al.* 2009) is compensated by quite gradual seed germination (Fig. 3). That is, even under continuous optimal water and temperature conditions, the species shows slow but progressive germination during a period of time equivalent to a whole season (3 months). *Ligustrum* cannot rely upon a seedbank, but instead has a ‘sparing’ germination strategy guaranteeing several cohorts of seedlings facing (or escaping) stochastic environmental constraints (e.g. exceptionally high temperatures or drought periods in summer). In contrast *Gleditsia* seeds can germinate almost 3 years after fruits have naturally fallen from the tree and remain viable probably until conditions for establishment are favorable (Ferrerías *et al.* 2015). Seed dispersal by animals (i.e. seed passage through the digestive tract of dispersers) does not increase seed germination of *Gleditsia* (Ferrerías *et al.* 2015), *Ligustrum* (Montaldo 1993) or *Pyracantha* (Badini 2012) but can certainly facilitate their spread in the field.

Overall, our findings show that seedlings of the three major woody invaders of low-mountain woodlands in central Argentina have the potential to establish and survive at higher elevations than those in which they currently occur. In contrast to woody natives, they seem to be broad climate tolerators, rather than specialized stress tolerators (see also Tecco *et al.* 2010, 2013; Zeballos *et al.* 2014b), capable of germinating and growing across a wide elevational range. This is consistent with the ‘directional

ecological filtering' hypothesis (*sensu* Alexander *et al.* 2011) that allows them to succeed as a 'jack-of-all trades' (i.e. maintain fitness in unfavorable conditions) (Richards *et al.* 2006). It is worth noting that these conclusions are fully grounded on an early life-cycle stage of the invaders. Whether climatic harshness affects other life cycle stages of these species (e.g. reproduction) or not, remains to be tested and claims for further long-term experiments. The inclusion of experimental tests for biotic limitations of competition, herbivory by invertebrates and grazing would also be necessary to comprehensively predict their potential for upper range expansion in these highly disturbed mountains (Cingolani *et al.* 2013, Cingolani *et al.* 2014).

### Mountain invasions on the way

Four main factors have been proposed to influence plant invasions into mountain ecosystems (Pauchard *et al.* 2009 and references there in). Specifically, upper range expansion of lowland invaders appears to be globally constrained by increasing climatic severity and by decreasing human disturbance and propagule pressure. Biotic resistance, though less studied, would apparently not limit altitudinal invasion, since the competitive interactions that prevail at lower elevations are expected to change towards facilitative interactions at higher elevations (Callaway *et al.* 2002). We suggest that the shape and relative importance of these factors are likely to vary among mountain ecosystems with different climates and land uses. For instance, in the Sierras Grandes mountains, subjected to a temperate/mesothermal climate with dry winters, (i) climatic severity would be least restrictive at mid elevations (see above); (ii) human disturbance would be high all across the range (i.e. ~400 years of livestock rearing with fire as rangeland management, Cingolani *et al.* 2013) particularly at mid elevations (Marcora *et al.* 2013); but (iii) the decreasing propagule pressure would be temporally restricting invaders to lower elevations (Giorgis *et al.* 2011a) as in global patterns. If true, the interaction of these drivers would place mid elevations, currently devoided of native forests (Marcora *et al.* 2013), as the most favorable belt for woody invasions once propagule pressure has been overcome. We lack of data on (iv) biotic interactions along the gradient to size up the influence of biotic limitations. For instance, positive interactions such as nursing (e.g. Cavieres *et al.* 2005, 2008) could also foster upper range expansion of these woody invaders (Tecco *et al.* 2006, 2007), at least up to 1700 m asl where *Pyracantha* is already established. So far, while this conceptual model remains to be studied, the existing observational (Giorgis *et al.* 2011a) and experimental (this study) evidences on woody invasions in these mountain range, seem to place propagule pressure, rather than climate, as the main constraint of woody invasion in the mountains from central Argentina.

### SUPPLEMENTARY DATA

Supplementary material is available at *Journal of Plant Ecology* online.

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