Do animal–plant interactions influence the spatial distribution of Aristotelia chilensis shrubs in temperate forests of southern South America?

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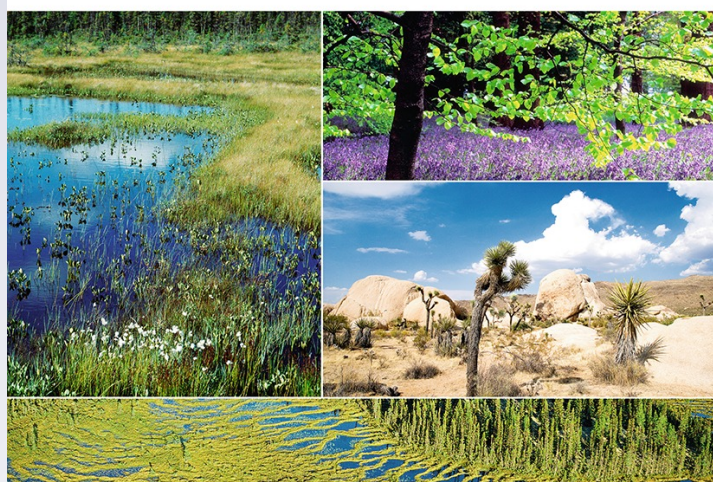# Do animal–plant interactions influence the spatial distribution of *Aristotelia chilensis* shrubs in temperate forests of southern South America?

**Susana P. Bravo · Victor R. Cueto ·
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Abstract Seed dispersal constrains the environmental heterogeneity to which a plant species is exposed through its life. Behavior of seed dispersers and seed predators could be influenced by food availability and vegetation cover. Consequently, recruitment probabilities are heterogeneous in space and time, and "regeneration windows" may appear. *Aristotelia chilensis* is the most abundant fleshy-fruited shrub in the temperate forest of southern South America (TFSA). TFSA exhibits an environmental patchiness that could influence fruit abundance and animal behavior. Our objective was to determine the regeneration windows of *A. chilensis*, and to detect how forest structure and animal behavior could affect the spatial distribution of this species. We characterized forest structure in two plots that included mature forest, young forest, and open areas. We assessed the spatio-temporal distribution of *A. chilensis* fruits and studied fruit removal by animals. Also, we experimentally evaluated germination and post-dispersal seed predation. We developed a conceptual model to relate *A. chilensis* recruitment with forest successional stages. The fruiting individuals of this shrub were mostly located in fire-opened areas or forest gaps, and the seed rain generated by the migratory bird *Elaenia albiceps* was denser in these areas. In contrast, seed predation by rodents was higher in closed, young forest areas. *A. chilensis* recruitment follows a nucleation dynamic around fruiting females. Concerning forest succession, *A. chilensis* recruitment was higher near re-sprouting females in open areas, and lowest in young forests, where fruiting process was hindered by light constraints. When forests mature, new nucleation processes start around females surviving in gaps. We conclude that areas opened by disturbances provide a regeneration window for *A. chilensis* shrubs.

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Introduction

Seed dispersal may constrain the environmental heterogeneity to which a plant species is exposed

through its life cycle (Snyder and Chesson 2003; Gómez et al. 2004). The majority of woody plants are dispersed by frugivorous animals (Herrera 2002). Seed dispersal has important ecological, evolutionary, and conservation implications for both the animal and plant species involved, as well as for the ecosystems in which it occurs (Herrera 2002; Schupp et al. 2010).

Seed rain generated by vertebrates commonly shows a heterogeneous spatial pattern, characterized by the presence of seed rain "hotspots" where a large number of seeds arrive (Schupp et al. 2010). Seed rain hotspots are determined by the spatial behavior of the disperser (e.g., the use of sleeping sites, perches, and latrines), which in turn is mostly affected by protection and distribution of food, among others (Carlo and Morales 2008; Hampe et al. 2008; Giombini et al. 2009; Bravo 2009; Morales et al. 2013). The spatial pattern of seed rain hotspots could be determined by feedback between the spatial distribution of fruiting plants and the behavioral responses of frugivores to these patterns (Aukema 2004). In particular for birds, mechanistic models predict that forest cover and fruit abundance modify bird behavior, and as a consequence affect seed rain (Morales et al. 2013).

Behavior of seed predators and environmental conditions of microsites may modify spatial patterns of recruitment by creation of spatial templates of seed survival probabilities different from the seed rain distribution. Spatial use by seed predators is influenced by food distribution and predation risk. Predation risk is commonly associated with high vegetation cover at low strata (e.g., for rodents, Drickamer 1990; Kotler et al. 1991; Dickman 1992). The effects of animal dispersers and predators may cancel out, because seed rain hotspots may be attractive areas with a high food concentration for seed predators and consequently, there may be low seedling recruitment. Furthermore, seedlings growing in a hotspot could suffer high density-dependent mortality. Despite these circumstances, high seed arrival rates may override mortality factors, allowing plants to recruit within seed rain hotspots, which in turn become "recruitment hotspots" (Hampe et al. 2008; Bravo 2012).

Consequently, in order to evaluate the role seed dispersal plays in driving plant distribution patterns and the spatial extent over which plant population processes operate, it is necessary to integrate information about behavior of seed dispersers and seed predators, and their relationships with environmental conditions, such as plant cover or resource distributions.

In the temperate forests of southern South America (hereafter, "TFSA"), 60 % of plant genera produce fleshy fruits (Aizen and Ezcurra 1998); however, the frugivore assemblage is reduced (Armesto et al. 1987; Aizen et al. 2002). There are 15–20 species that are partially frugivorous, and birds represent the most relevant group (Aizen et al. 2002). However, only two bird species (*Elaenia albiceps* and *Turdus falckandii*) are potentially true seed dispersers because of their relatively high abundance and the high frequency of fruits found in their diets (Willson 1991; Aizen et al. 2002). In particular, during summer, the small migrant *E. albiceps* is the most abundant species of the bird assemblage (Grigera et al. 1994; Ippi et al. 2009), and the main frugivore in the TFSA (Amico and Aizen 2005; Cavallero et al. 2012).

Aristotelia chilensis (Elaeocarpaceae) is one of the more abundant fleshy fruit species in northern TFSA; it produces fruits during mid-summer (Amico and Aizen 2005; Garcia et al. 2011) just before the migration of *E. albiceps* to their wintering areas in the tropics (Fitzpatrick 2004). Although *A. chilensis* produces large crops of fleshy fruits and large seeds in relation to other plant species of the TFSA, the influences of seed dispersal and seed predation on its spatial distribution have been scarcely studied (Garcia et al. 2012). Environmental conditions, especially light availability, have been proposed as determinants of the spatial distribution of *A. chilensis* (Lusk and del Pozo 2002; Salgado-Luarte and Gianoli 2011), because high light conditions increase germination and seedling growth, and decrease plant mortality during the first year (Lusk and del Pozo 2002; Figueroa 2003).

The TFSA exhibits an environmental patchiness that could influence animal behavior and fruit abundance through changes in vegetation cover that affect predation risk and light availability in the understory. This spatial heterogeneity is a consequence of a disturbance regime mainly driven by fire, tectonism, wind, snow avalanches, and tree falls (Veblen et al. 1992; Kitzberger et al. 1995, 1997). Regeneration of open areas occurs through germination of seeds from survivor trees or from adult neighboring trees (Premoli and Kitzberger 2005), resulting in closed forests of even-aged young trees with a high accumulation of litter (Veblen and Lorenz 1987; Veblen et al. 1992). Over time, some trees die, decreasing forest density and facilitating the growth of surviving individuals (Veblen et al. 1992). In older forests, the generation of

gaps produced by the breakage of large branches is frequent, such that a new cycle begins after each strong disturbance (Veblen et al. 1992). We predict that heterogeneity may have a reinforcing role on *A. chilensis* recruitment. Recruitment hotspots for *A. chilensis* would be located in exposed areas created by disturbance, because they would have high fruit production and consequently, they would have high seed rain and low predation rates (due to low vegetation cover), whereas young forests will have low seed rain and high seed predation rates. Thus, dispersal and predation may reinforce the prevalence of *A. chilensis* in open areas.

We studied the seed rain of *A. chilensis* in the TFSA in order to determine if seed rain produced by birds occurs in hotspots, and to determine if forest structure and fruit abundance influence the spatial distribution of seed rain hotspots. We also studied whether there is differential seed predation that can influence the formation of recruitment hotspots of *A. chilensis* in open areas, as well as forest heterogeneity and distribution of fruits in time and space. This evaluated the contribution of birds to seed removal from shrubs, and from the bird's perspective, the relevance of *A. chilensis* fruit in their diet. We monitored shrubs to detect the presence of mammalian seed dispersers and characterized the spatial pattern of seed rain by searching for hotspot areas and quantifying their relationship with environmental heterogeneity and fruit distribution. Finally, we studied the germination of seeds dispersed by *E. albiceps* compared to non-dispersed seeds, and the risk of seed predation in relation to forest heterogeneity. Using the obtained data from this study, we propose a conceptual model of the relationship between *A. chilensis* recruitment and forest succession to determine the regeneration window (Kollmann 1995) of *A. chilensis* in the TFSA.

Methods

Study site

The study was carried out in a mixed *Nothofagus dombeyi* (Nothofagaceae) and *Austrocedrus chilensis* (Cupressaceae) forest on the northern margin of Lake Steffen (41°31′ S y 71°35′ W, 550 m a.s.l.), Nahuel Huapi National Park, northern Patagonia, Argentina, between November and April (spring, summer, and autumn in the southern hemisphere), during three consecutive years (2009–2010, 2010–2011, and 2011–2012). Vegetation corresponds to the Subantarctic Biogeographic Province (Cabrera and Willink 1980). The forest canopy is dominated by *N. dombeyi* with some *A. chilensis*. The most representative species of the understory are *A. chilensis*, *Schinus patagonicus* (Anacardiaceae), *Berberis* spp. (Berberidaceae) and *Lomatia hirsuta* (Proteaceae). *A. chilensis*, *Berberis* spp., and *S. patagonicus* all produce fleshy fruit. In general, bamboo is a dominant species in the TFSA understory; however, it is rare in the study area.

The climate is cold-temperate and precipitation is concentrated during autumn and winter (April–September). At Lake Steffen, the annual mean precipitation is 1,274 mm (1993–2011, Station No. 2300 "Lago Steffen", Red Hidrológica Nacional, Subsecretaría de Recursos Hídricos de la Nación). The annual mean temperature is 8.5 °C, the maximum mean temperature (January) is 16.2 °C, and the minimum mean temperature (July) is 2.2 °C (2000–2010).

Two plots were established in the deltas of the two biggest streams on the northern margin of Lake Steffen. The plot areas were 6 ha (Plot 1) and 4 ha (Plot 2), and each one was divided into squares 25×25 m (hereafter, "subplots"). Plots 1 and 2 were composed of 95 and 64 subplots, respectively. These zones were the only relatively accessible, well-preserved humid areas with mixed forest, low slopes, a low presence of livestock, and no presence of exotic ungulates in the region around Lake Steffen.

Forest heterogeneity

Three different types of patches were considered: open areas, young forest, and old forest. We mapped the presence of these three patch types in each plot and measured the vegetation structure and composition of 24 subplots (a total of 8 subplots/patch type), randomly located in each patch type of Plots 1 and 2. In the center of each subplot, we established a 10-m transect in each of the four cardinal directions. We sampled vegetation at 20 random points along each transect by erecting a 10-m-tall rod marked at 0.5-m intervals and recording the height and species of vegetation in contact with the rod. Considering the distribution of rod contacts in all subplots, we defined four strata: 0–0.5, 0.5–5, 5–10, and >10 m. In each stratum, the horizontal cover of each species was

calculated as the percentage of points where the species was present. Total vegetation cover for each stratum was calculated as the percentage of points where any plant was present. We estimated a mean value over the four transects for each of the 24 subplots in each plot. We randomly selected five subplots in old forest and five in young forest, where we recorded the number of *N. dombeyi* trees and measured their diameter to breast height (DBH).

The abiotic characterization of the three patch types was evaluated through measures of light and temperature at 20 cm above the soil surface. Measurements were recorded every half hour from January to April 2012 using Hobos pendant temperature/light data loggers UA 002-08 (Onset Computer Corporation). We selected two random points in each patch type to record these abiotic variables and estimated a mean value over the two points for each patch type.

Aristotelia chilensis fruit distribution in time and space

To evaluate within growing season variation in ripe fruit abundance, we estimated weekly the quantity of mature fruits on 20 individual shrubs (10/plot) using a semi-quantitative index from 0 to 5 (Saracco et al. 2004), where 0: without ripe fruits, 1: 1–10 ripe fruits, 2: 11–50 ripe fruits, 3: 51–100 ripe fruits, 4: 101–500 ripe fruits, and 5: 501–1,000 ripe fruits.

The cover of *A. chilensis* in each stratum was recorded during the evaluation of forest heterogeneity described in the previous section. However, as *A. chilensis* is a dioecious species, we evaluated the abundance of females to estimate fruit abundance in plots. We evaluated their spatial distribution and searched for any relationship with environment heterogeneity. During spring/summer 2011/2012, the spatial distribution of *A. chilensis* females was determined by mapping all flowering and fruiting females in both plots. Furthermore, in all subplots of the old forest where fruiting females were found, we evaluated the canopy cover (>10 m) using the same method described in the previous section.

Frugivory from the bird's perspective

From November 2010 to March 2011, we captured birds every 15 days in each plot using 12 mist nets (12 m long, 3 m high, 38-mm mesh) separated 70–100 m from each other. During two consecutive days, nets were opened and all captured birds were retained for up to an hour in bags, or until fecal samples were obtained. In the laboratory, each fecal sample was disaggregated, the number of *A. chilensis* seeds found was recorded and the bird species that produced the sample was noted.

Frugivory from the plant's perspective

During the peak of fruit production, six shrubs with a phenological category of 4 or 5 were monitored by infrared camera traps with motion sensors (Cudeback Noflash and Moultrie I40). These shrubs were monitored during three consecutive day–night periods, and the number of visits of every species (birds and mammals) that arrived to eat fruit was recorded.

Spatial pattern of seed rain

During the summer of 2011, 72 seed traps were placed every 50 m in both plots, 49 in Plot 1, and 23 in Plot 2. Each seed trap consisted of four squares of 0.5 × 0.5 m of 1-mm nylon mesh, suspended 0.3 m above the ground on four wire poles. From 1 February to 15 March, traps were visited every 2 weeks. Seeds found in each trap were removed and stored in paper bags. In the laboratory, the samples were cleaned and *A. chilensis* seeds were counted under a dissecting microscope.

Seed germination

In order to determine if passage through the digestive tract of *E. albiceps* affects germination, we measured germination of dispersed and non-dispersed seeds. We collected 260 seeds from *E. albiceps* fecal samples and 260 seeds from ripe fruits collected from five shrubs. Seeds collected from the shrubs were manually separated from the pulp. Seeds were placed in Petri dishes, 52 seeds/dish and 5 dishes/treatment, on filter paper. Dishes were moistened with distilled water every 2 days and maintained in a cycle of 10:14 light:dark hours, at 20 °C during the light period and at 5 °C during darkness. We evaluated three germination parameters: percentage of seeds germinated after 30 days without new germinations, number of days to germination of the first seed (initial time), and number of days at which 50 % of seeds were germinated (T_{50}). We considered every Petri dish as a replicate.

Seed predation

In order to evaluate if the next step of the seed dispersal cycle could also be influenced by forest heterogeneity, in the summer of 2012 we measured seed predation in the three forest patch types. Experiments were carried out in Plot 1 because it better represented the spatial heterogeneity of the forest. Thirty dishes with 10 seeds of *A. chilensis* were placed in each patch type during three consecutive day–night periods. Dishes were randomly located at 25 m from each other and the number of seeds removed or predated in situ was recorded. Predation in situ was determined by the presence of seed-coat fragments in the dishes. Five infrared camera traps with motion sensors were located at five experimental stations to identify seed predators.

Statistical analysis

Because only a low number of subplots correspond to open areas in Plot 2 (see "Results" section), we analyzed the forest structure of both plots together. The cover of *A. chilensis* in each forest patch type was compared using One Way ANOVA and a posteriori Tukey comparisons. The stratum cover in each patch was evaluated using Two Way ANOVA. Because there was no canopy stratum in open areas, we had unequal numbers of replicates per cell, so we used the type-III method to compute sum of squares (Hector et al. 2010). Comparisons of stratum cover among treatments, open areas, young forest, and old forest, were performed using the Kramer modification of Tukey's test (Day and Quinn 1989).

We analyzed the spatial distribution of *A. chilensis* fruiting females and seed rain at different scales using principal coordinates of neighbor matrices (PCNMs; Borcard and Legendre 2002, 2004). The PCNM method creates a set of spatial explanatory variables (vectors) that have structure at all scales encompassed by the data matrix and determines to which of these variables the response data are statistically responding (Borcard and Legendre 2002; Borcard et al. 2004). Starting from the X and Y Cartesian coordinates system, a distance matrix was created using Euclidean distance and the simple linkage method. The matrix was truncated at the minimal distance to allow all the points to be graphed and connected. With this new matrix, we performed principal component matrix analysis. The number of PCNM vectors was reduced by computing a linear regression with a backward stepwise selection process, using PCNM vectors as predictor variables. Remaining vectors were divided into three groups to obtain the models at each scale (broad, meso, and fine). As our data were not obtained by regular sampling lines, PCNM vectors did not display a regular sine-shaped pattern; however, the progression from a broad to a fine scale was preserved. We tested the significance of each scale-model and vectors in the model by computing regressions and using backward stepwise selection. For more detailed information about the PCNM method, see Borcard and Legendre (2002) and Borcard et al. (2004). PCNM-predicted values of seed rain were correlated with raw values of *A. chilensis* fruiting females, using a Spearman's correlation.

Searching for robusticity in the results, and in order to determine the size of seed rain hotspots, we evaluated the association between fruiting females and seed rain distribution using a Bivariate version of the O-ring statistic (Wiegand and Moloney 2004), which is similar to Ripley's K, but uses rings instead of circles. The O-ring statistic is based on counting the number of points of pattern 2 at distance r to the points of pattern 1. Thus, the focal point is not counted. Values of the O-ring statistic higher than the critical value indicate that there are more points of pattern 2 within a radius r to points of pattern 1 than expected by chance, indicating an attraction between the two patterns at distance r. Regarding our biological hypothesis, we used a null model of antecedent condition because the creation of pattern 2 (seed rain) might be influenced by pattern 1 (fruiting females; Wiegand and Moloney 2004). Hence, the positions of fruiting females were held fixed and the positions of seeds were changed randomly. To determine the statistical significance of the association between both patterns, confidence envelopes for the O_{12}-ring statistic were obtained after 99 Monte Carlo simulations based on a heterogeneous Poisson distribution, because PCNM analysis detected the existence of a non-homogeneous pattern. We performed statistics and simulations with the free software Programita (Wiegand and Moloney 2004).

We evaluated differences in percentage, T_{50} and initial time of germination for dispersed and non-dispersed seeds of *A. chilensis* with a Student t test. The number of seeds removed and the proportion of seeds predated in each patch type of the forest were evaluated through One Way ANOVAs and a posteriori Tukey comparisons.

Results

Forest heterogeneity

Plot 1 was more heterogeneous, with the three forest patch types well represented (48 % young forest, 32 % old forest, and 20 % open areas), while in Plot 2 young forest was the main patch type (72 % young forest, 22 % old forest, and 6 % open areas). In old and young forest patches, the canopy was dominated by *N. dombeyi*. The DBH of *N. dombeyi* (pooling data of both plots) in the old forest patch was 57.4 ± 10.6 cm (mean $\pm$ SD) and the number of individuals was 397 ± 329 ind/ha (mean $\pm$ SD), while in the young forest, the mean DBH was 15.0 ± 4.8 cm and the number of individuals was $1,910 \pm 1,348$ ind/ha.

In the young forest, we recorded a lower thermal amplitude (7 °C), and the lowest light intensity, with 24 % of light received in open areas. Open areas had the highest thermal amplitude (23.5 °C) and the maximum light intensity. In old forest, we recorded intermediate values for both parameters, where thermal amplitude was 11.5 °C and light intensity was 48 %.

The three forest patches differed in their vertical structure (Fig. 1; interaction forest patch-stratum; $F_{6,84} = 8.83$, $P < 0.0001$). Young forest had the highest cover values in the canopy and lower strata, and the lowest values at the intermediate strata (5–10 m), while in open areas the upper stratum (>10 m) was absent and showed the lowest cover between 5 and 10 m. Old forest had the lowest cover in the lower strata (0–0.5 m).

Three fleshy fruit species were the most common in both plots, but *A. chilensis* was the dominant species in the understory of the forest (Table 1). Total cover of *A. chilensis* was significantly higher in young forest than in the other two patch types (Table 1; $F_{2,21} = 8.59$, $P = 0.002$); however, we observed that most individuals in the young forest had a high proportion of dead branches and few leaves.

Fruit distribution

Ripe fruits of *A. chilensis* were available from the last week of January to the first week of March, with the peak of production around mid-February. We were unable to determine the sex of all shrubs, since not all shrubs flowered. PCNM analysis revealed that fruiting females
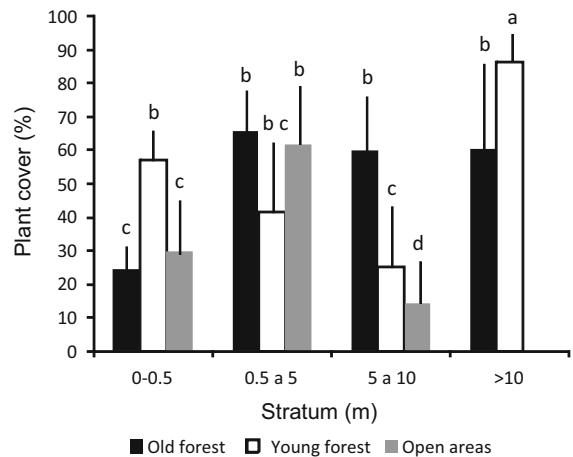

Fig. 1 Vegetation cover of different strata in the three patches of mixed *Nothofagus dombeyi* and *Austrocedrus chilensis* forest near Lake Steffen, Nahuel Huapi National Park, Argentina. *Bars with the same letter* are not significantly different from each other according to the Kramer modification of Tukey's test ($P < 0.05$)

Table 1 Percentage of cover (mean $\pm$ SD) of the most abundant fleshy fruit species in three patch types of *Nothofagus dombeyi* and *Austrocedrus chilensis* mixed forest near Lake Steffen, Nahuel Huapi National Park, Argentina

Forest patches	*Aristotelia chilensis*	*Schinus patagonica*	*Berberis* spp.
Young forest	34.4 ± 5.8	6.5 ± 0.6	0.6 ± 0.6
Old forest	13.1 ± 3.1	8.8 ± 0.9	2.5 ± 0.9
Open areas	11.7 ± 2.8	8.3 ± 2.8	4.4 ± 1.8

have a nonrandom spatial distribution at the scale of the study area (i.e., broad scale). From the 24 vectors generated during the analysis for Plot 1, only 15 were significant; ultimately, the broad-scale sub-model, retaining only 4 vectors, accounted for 67 % of the spatial variance (Table 2). Of the 28 vectors generated for Plot 2, 22 were significant, and only the broader scale sub-model, retaining 3 vectors, accounted significantly for 80 % of the spatial variance (Table 2). In both plots, fruiting females exhibited the lowest abundance in the younger forest (Plot 1 + Plot 2 = 10 individuals), while the old forest (Plot 1 + Plot 2 = 41 individuals) and the open areas (Plot 1 + Plot 2 = 45 individuals) had an aggregated distribution of fruiting females (Online Resource 1). Subplots in the old forest with fruiting females had lower canopy cover than the average cover for this type of forest (mean $\pm$ SD 13.6 ± 14.3 and 60.2 ± 34.8 %, respectively).

Table 2 Summary of multiple regression models fitting fruiting female abundance and seed rain distribution to principal coordinates of neighbor matrix (PCNM) vectors in Plots 1 and 2 of *Nothofagus dombeyi* and *Austrocedrus chilensis* mixed forest near Lake Steffen, Nahuel Huapi National Park, Argentina

Scales	Fruiting females				Seed rain			
	No. of vectors		R^2		No. of vectors		R^2	
	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2	Plot 1	Plot 2
Broad	4	3	0.67***	0.8*	2	3	0.95***	0.76**
Meso	1	7	0.18 NS	0.06 NS	3	4	0.03 NS	0.01 NS
Fine	1	7	0.05 NS	0.02 NS	2	4	0.01 NS	0.12 NS
Total			0.90***	0.88*			0.99***	0.89**

NS non-significant

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0001$

Frugivory

We captured 342 *E. albiceps* with mist nets, which represented 88 % of captures, and only 10 individuals of another frugivorous species (*T. falckandii*), representing 3 % of captures. A total of 112 fecal samples of *E. albiceps* containing seeds of *A. chilensis* were obtained. The first *A. chilensis* seeds of the season were found in the third week of January, representing 31.6 % of samples (1–5 seeds/fecal sample). The last fecal samples with seeds were obtained in the first week of March, representing 63.6 % of samples (1–8 seeds/sample). During the peak of fruit production, 95 % of samples had 1–21 seeds, and 4 % of samples did not have any insects.

Sixty-three camera trap photographs indicated that 100 % of visits to feed on *A. chilensis* fruits corresponded to *E. albiceps*. Most photographs were taken between 13:00 and 18:30 h, which coincided with maximum daytime temperatures. No mammals were photographed.

Seed rain

More than 90 % of seeds found in seed traps were *A. chilensis* seeds. In Plot 1, PCNM analysis results indicated seed rain had a nonrandom spatial distribution at the broad scale of the study. From eight generated vectors, only two were included in the broad-scale sub-model, accounting for 95 % of the spatial variance (Table 2, Online Resource 2a, b). In Plot 2, PCNM analysis produced similar results. Seed rain had a nonrandom spatial distribution at the broad scale of the study. From 13 generated vectors, only 3 were included in the broad-scale sub-model, accounting for 76 % of spatial variance (Table 2, Online Resource 2c, d).

Seed rain showed a similar pattern as fruit availability, with a low intensity in young forest (mean ± SD; Plot 1, 4.2 ± 2.6 seeds/trap; Plot 2, 1.6 ± 0.7 seeds/trap), and an aggregated pattern in old forest (mean ± SD; Plot 1, 26.6 ± 33.6 seeds/trap; Plot 2, 6.4 ± 4.8 seeds/trap) and open areas (mean ± SD; Plot 1, 36.2 ± 31.4 seeds/trap; Plot 2, 6.0 ± 2.8 seeds/trap). Both PCNM analyses (Plot 1, $r_s = 0.55, P < 0.0001$; Plot 2, $r_s = 0.77, P < 0.0001$) and the point pattern analysis (Fig. 2) indicated that seed rain hotspot areas were associated with zones of high abundance of fruiting females. The O-ring values along a gradient of distances between seed traps and fruiting adults indicated a significant and positive correlation between females and dispersed seeds at short distances, suggesting that seed rain hotpots occur within the close neighborhood of female shrubs. More seeds were dispersed in Plot 1 than in Plot 2 (945 vs. 57 seeds, respectively), and this was reflected in hotspot size. In Plot 1, seed rain hotspots occurred in the subplot with fruiting females (0 m), and within the two contiguous subplots it was a distance of up to 50 m (Fig. 2a). In Plot 2, seed rain hotspots occurred in the quadrant with fruiting females and only within the first contiguous subplot, up to 25 m (Fig. 2b).

Germination

Dispersed seeds from *E. albiceps* fecal samples had a similar germination percentage than non-dispersed seeds collected from *A. chilensis* fruits (dispersed 9.6 ± 5.2 %, non-dispersed 10.9 ± 6.1 %, $t_8 = 0.15$,
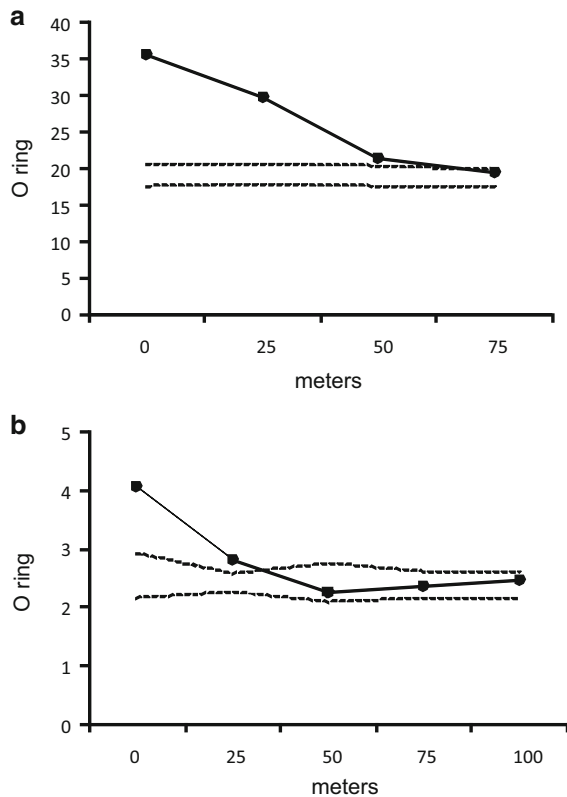
Fig. 2 O_{12}-ring values for seed rain at different distances of fruiting females for Plot 1 (**a**) and Plot 2 (**b**) in mixed *Nothofagus dombeyi* and *Austrocedrus chilensis* forest near Lake Steffen, Nahuel Huapi National Park, Argentina. *Pointed lines* indicate confidence intervals, higher values indicating an association between females and dispersed seeds (for further details, see "Methods" section)

$P = 0.88$). Additionally, germination speed was similar for the two groups in terms of the initial time (dispersed 43.3 ± 13.9 days, non-dispersed 38.9 ± 6.6 days, $t_8 = 0.64$, $P = 0.5$), and T_{50} (41.5 ± 14 days, 38.9 ± 6.6 days, $t_8 = 0.84$, $P = 0.4$).

Seed predation

Seed loss was mainly due to predation in situ. Camera recordings indicated that all seed predation events were during the night, and that small rodents were responsible for seed predation and removal during experiments. The proportion of predation and removal were different among forest patch types (predation: $F_{3,88} = 4.16$, $P = 0.02$, Fig. 3a, removal: $F_{3,88} = 3.69$, $P = 0.04$, Fig. 3b). Seed predation and removal were higher in

young forest than in old forest and open areas (Tukey test, $P < 0.05$).

Discussion

Seed rain hotspots of *A. chilensis* generated by the frugivorous bird *E. albiceps* are associated with zones of fruiting production. Similar patterns were described for bamboo-dominated old forest in the TFSA and Cantabrian forest in Spain (Garcia et al. 2011). These results confirm the patterns predicted by some mechanistic models, that forest cover and fruit abundance modify bird behavior, and as a consequence affect seed rain (Morales et al. 2013). Seed rain intensity was higher where fruit production was also higher, namely in open areas and gaps in old forest. This was also true at the plot scale where Plot 1 had more fruit production and consequently higher seed rain intensity relative to Plot 2. *E. albiceps* was the main seed disperser of *A. chilensis* at our study site, which is consistent with previous reports for other forests in the TFSA (Amico and Aizen 2005).

As predicted, the highest seed predation occurred in young forest characterized by the highest cover of the low stratum (90 % of which is composed of a dense litter including dead branches and twigs). Therefore, the forest patch type with the fewest seeds arriving also had the highest rate of seed predation. High predation of seeds and seedlings by rodents in areas with dense understory is a common pattern observed in the TFSA and in other ecosystems; consequently, exposed zones (e.g., gaps and open areas) are good sites for regeneration (Wada 1993; Iida 2004, 2006; Caccia et al. 2009).

We recorded flowering and fruiting individuals only in exposed areas (i.e., gaps and open areas). Seed rain hotspots were associated with the spatial distribution of fruit. Therefore, the understudied flowering physiology of *A. chilensis* could be an important determinant in its recruitment pattern. The balance of environmental signals (light and temperature) and internal signals (hormones and autonomous pathways) is integrated and determines when flowering occurs in plants (Putterill et al. 2004). Plants detect overcrowding by changes in light quality (through the decrease in the ratio of red–far-red light) and can modify their growth or flowering phenology as a function of those signals (Schmitt et al. 1995; Putterill et al. 2004;
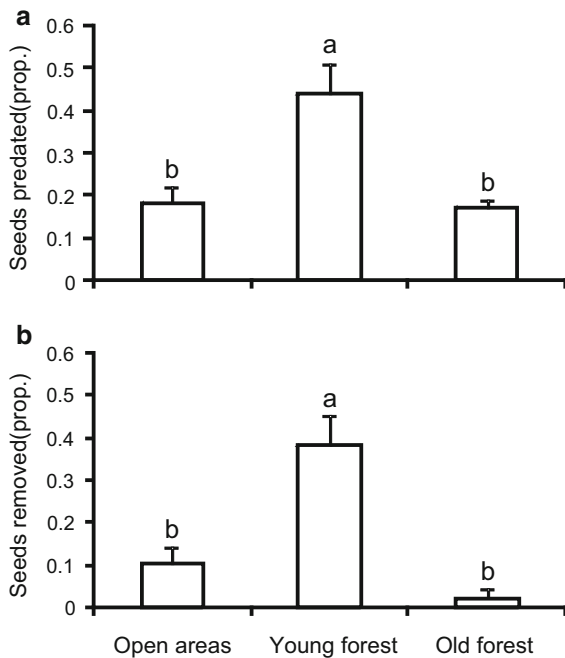
Fig. 3 Results of seed predation experiments in three patches of mixed *Nothofagus dombeyi* and *Austrocedrus chilensis* forest near Lake Steffen, Nahuel Huapi National Park, Argentina. **a** Seeds predated in situ, and **b** seeds removed. *Bars with the same letter* are not significantly different from each other according to Tukey's test ($P < 0.05$)

Franklin and Whitelam 2005). In conclusion, based on our results on the non-flowering of *A. chilensis* in young forests and partial flowering in old forest, we propose that a possible *A. chilensis* strategy (or the first sign of loss of vigor) in the face of overcrowding is to stop or decrease flowering, and that this is an important regulator of subsequent steps in the seed dispersal cycle.
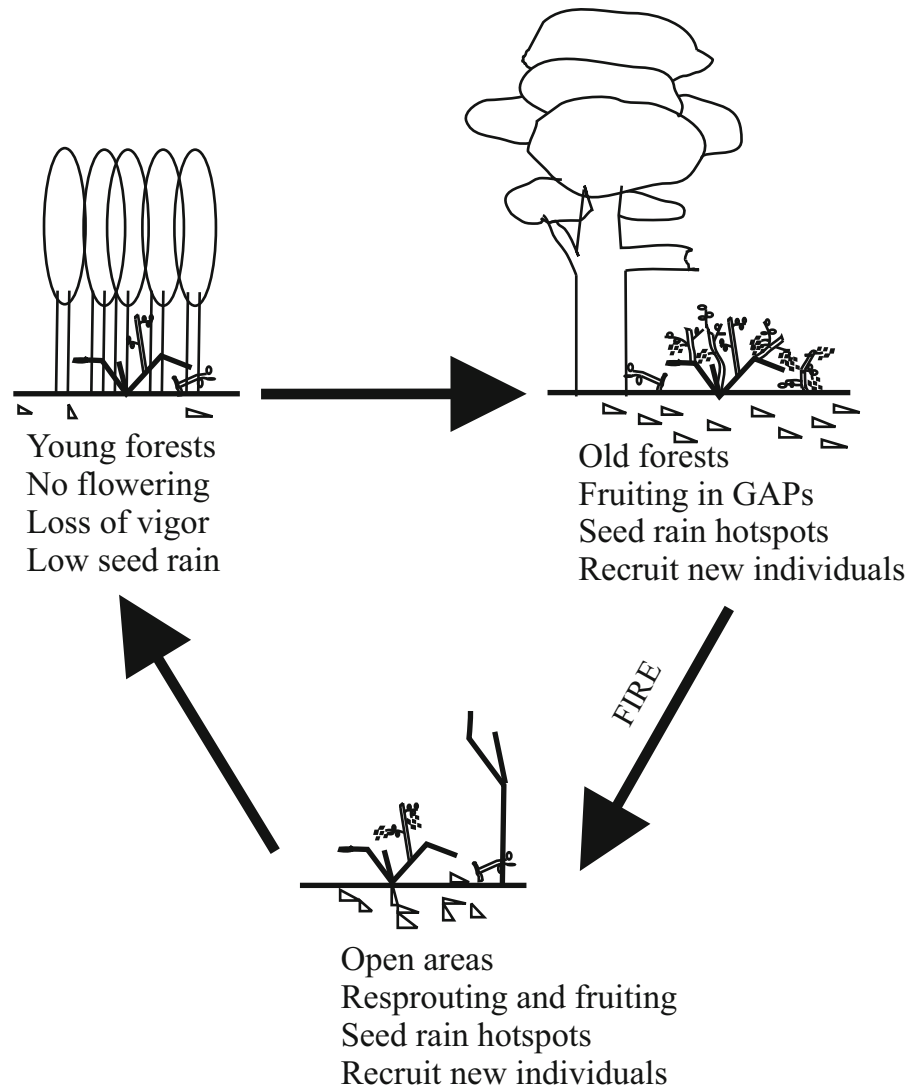
The transition from seeds to seedlings implies the survival of seeds until germination. According to our data, seed predation was low in exposed sites where seed rain hotspots were located but high in young forest where arrival of seeds was lowest; therefore, the probability of survival until germination in closed areas is low. Although our germination field experiment was suspended in the first year due to a "ratada" (extraordinary rodent population growth mediated by regional bamboo flowering), and the second year's experiment was canceled due to vandalism, all information available in the literature is consistent with our results, namely that high light conditions are necessary for germination and growth of *A. chilensis* seedlings

(Lusk and del Pozo 2002; Figueroa 2003; Salgado-Luarte and Gianoli 2011). In that context, germination and seedling physiology are not the determinants of *A. chilensis* recruitment in exposed areas, because seed rain and seed survival prevent seeds from arriving at sites with unfavorable conditions for germination and seedling survivorship.

The three forest patch types in our plots were representative of different successional stages of the TFSA (Veblen et al. 1992). In both plots, we found old burn signs on trunks of *N. dombeyi* trees, as well as old *A. chilensis* individuals that had been burned and which were re-sprouting and flowering in open areas. We propose a conceptual model on the role of *E. albiceps* in the recruitment of *A. chilensis* based on the forest dynamics observed at our study site (Fig. 4). The first step of the model occurs in an open area, where new individuals would be recruiting year after year near the location of re-sprouting fruiting females. This feedback between fruiting females and seed rain could end when open areas complete their transition to young forest environments, with a subsequent decrease in light in the understory, resulting in non-flowering, loss of vigor, and probably mortality of shrubs. Although we recorded higher cover of *A. chilensis* in young forest, most shrubs exhibited low vigor (i.e., few leaves per branch and several dead branches). A similar lack of vigor and small, dead individuals were observed in young forest in other areas of the TFSA (Kitzberger pers. com.; Lusk 2004). As a consequence of low fruit availability, the use of those areas by *E. albiceps* also decreases, and seed predation by rodents increases as a consequence of the dense understory; therefore, the probability of recruitment will be lowest in young forests (Fig. 4). Surviving females in mature forest could recover vigor and start flowering and fruiting again if a gap is created on a female's patch, attracting *E. albiceps* and creating a seed rain hotspot. This could result in the formation of a recruitment hotspot in a forest with low seed predation, increasing the recruitment probability of females in that spot (Fig. 4). A new fire event could return the system to the initial point of the model, with the most vigorous females re-sprouting after the fire.

The strong positive association between fruiting and light has been described previously, particularly for *A. chilensis* fruiting individuals associated with areas of low bamboo cover in more humid areas, where forest is dominated by bamboo in the understory (Veblen et al.

Fig. 4 Conceptual model of *A. chilensis* regeneration in the context of forest dynamics at our study site, the mixed *Nothofagus dombeyi* and *Austrocedrus chilensis* forest near Lake Steffen, Nahuel Huapi National Park, Argentina. *Triangles* represent dispersed *A. chilensis* seeds, and *arrows* represent time

Young forests
No flowering
Loss of vigor
Low seed rain

Old forests
Fruiting in GAPs
Seed rain hotspots
Recruit new individuals

FIRE

Open areas
Resprouting and fruiting
Seed rain hotspots
Recruit new individuals

1992; Garcia et al. 2011). On the western side of the Andes Mountain range, with an even more humid climate and higher bamboo cover, this strong relationship between light and *A. chilensis* fruiting has not been reported, because this species is limited only to high-light environments there (Lusk 2002, 2004). Therefore, mortality of *A. chilensis* in the presence of bamboo is probably higher, and long distance seed dispersal to arrive at large open areas would be more important in those situations than in areas without bamboo, such as at our study site.

Studies on the regeneration of the TFSA after fire in zones with different floristic compositions provide evidence that (1) *E. albiceps* is the most abundant frugivorous bird, and (2) regeneration follows a nucleation dynamic (Albornoz et al. 2013; Cavallero et al. 2013). Our conceptual model on *A. chilensis* recruitment agrees with those studies of forest regeneration after fire. In particular, we propose that after fire, surviving *A. chilensis* females start a nucleation process of regeneration through seed dispersal by *E. albiceps*, resulting in a patchy recruitment pattern. Our results highlight the influence of animal–plant interactions on the spatial distribution of plants, in particular the relevance of the functional role of migratory birds in the TFSA, and demonstrate that areas opened by disturbances and gaps in old forests of the TFSA are the regeneration window for *A. chilensis*.

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