Seed predation and its relationship with the recruitment of seedlings of woody species in central Chile

María José Correa-Solís Román

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Pontificia Universidad Católica de Chile
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María José Correa-Solís Román

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Thesis Committee

Pablo Becerra Osses, Advisor

Ramiro Bustamante

Cristián Bonacic

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To Dr. Luis Zúñiga, my first inspiration to study ecology.

.

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General Discussion

General Introduction

Regeneration is a critical process for the development of plant communities, succession processes and the planning of ecological restoration treatments (Grubb 1977, Crouzeilles et al. 2017, do Santos et al. 2019, Walker et al. 2007). Biotic and abiotic factors have a role in these processes, and may either hinder or promote the regeneration of woody plants. Among such characteristics, herbivory has been shown to have an important effect on seed bank, germination and seedling recruitment (Wenny 2000, Paine & Beck 2007, Vaz Ferreira et al. 2011, Larios et al. 2017).

Seed predation can have a significant impact on the structure and the dynamic of plant populations (Wenny 2000, Paine & Beck 2007, Vaz Ferreira et al. 2011, Larios et al. 2017). Taking into account that plants lose 90% to 100% of their seeds as a result of predation (Crawley 1992, Hulme 1998), it is generally considered that seeds are the most vulnerable state in the plant life cycle (Hanley et al. 2007). Similarly, the impact of seedling predation has been reported as an important mortality factor in regeneration (Harper 1977, Leck et al. 2008), affecting population dynamics and expansion (Körner 2003, Clark et al. 2007).

The effects of herbivory on seeds or seedlings may depend on a number of factors. In the first place, the plant species and the herbivore assemblage present on site may affect the level of herbivory. The herbivore assemblage may vary in terms of diversity and abundance, between habitat or microhabitat conditions, and between their different geographical location (Crawley 1992). Notably, predation effects on seeds and recently germinated seedlings are critical when they occur in habitat and microhabitat conditions, where abiotic conditions are most favorable for species recruitment and regeneration (Harper 1977, Grubb 1977).

In semi-arid ecosystems, it has often been reported that in all probability the regeneration of woody species takes place in microhabitats with shrub or tree canopy characteristics (Maestre et al. 2003). Higher levels of moisture in the soil, nutrient availability and the reduced thermal oscillation generated under the canopy, would make this possible (Cerda 1997, Bochet et al. 1999, Murchie & Horton 1998, Valladares & Puignaire 1999, Maestre et al. 2001).

In semi-arid regions where forest ecosystems have been degraded, vegetation normally appears as a patchwork mosaic of woody plants, with different cover and height levels (Fuentes et al. 1986, Schulz et al. 2010). There is also greater abundance, seedling survival and growth in woody patches (Whittaker & Levin 1977, Peterson & Picket 1990, Peterson & Campbell 1993). Furthermore, bush patches act as nurse plants that aid in the regeneration of mid or late-succession species, improving the progress and recovery of advanced succession stages (García-Fayos & Verdú 1998, Jordano 2000, Maestre et al. 2003). However, in degraded ecosystems that initially consisted of forests, the greatest number and variety of herbivorous seed and plant predators, are mostly found in patches of woody species with greater coverage and height (e.g. Díaz et al. 1999, Kelt et al. 2004). It is therefore likely, that most herbivory happens precisely in such microhabitats that contain the best abiotic conditions for plant germination and survival. However, this may depend considerably on micro habitat preferences, and herbivores' specific locality or region. For instance, in rodents, that are mainly granivores, it has been described that as anti-predator behavior, they show a preference for vegetation cover as refuge (Guiden & Orrock 2017, Wang et al. 2019).

In summary, vegetation patch effect on the regeneration of woody species may occur through at least two types of relationships, one positive and the other negative (Fig. 1).

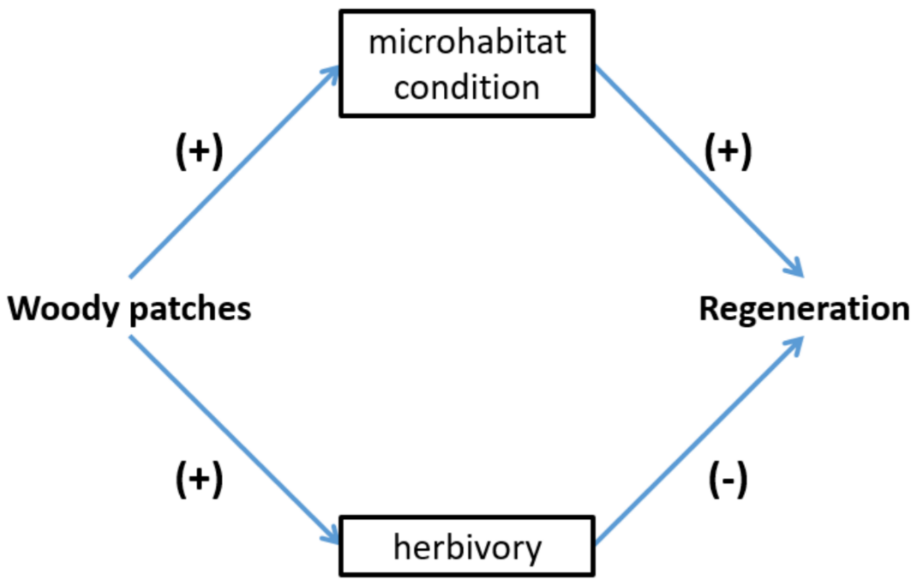

Figure 1. Relationship between the vegetation patch and regeneration mediated by abiotic conditions and herbivory in arid and semi-arid environments. The abiotic effect of patches is positive, however, as a result of herbivory the effect could be negative.

Hence, the effect that vegetation patches have on regeneration, will offer a net result generated by the combined effect of abiotic and biotic effects associated with herbivory. Additionally, herbivory effects on regeneration will be the result of herbivore effect on seed predation, and the effect of herbivory on plants (Harper 1977).

It is a generally known fact that in semi-arid ecosystems, there is a positive effect, through the enhanced development of abiotic conditions for seedlings, on the regeneration of patches with greater woody cover (bush and tree; Maestre et al. 2009). However, there is less information available with regard to the role and importance of vegetation patch type, in modulating the level of herbivory.

Different methods such as sowing are an important approach for reforestation and ecosystem restoration, and may be the only form of reforestation in remote and inaccessible areas (Woods & Elliott 2004, Doust et al. 2006, Grossnickle & Ivetic 2017). Likewise, in diverse environments, natural regeneration is the main strategy to recover and restore ecosystems. Therefore, it is relevant to establish whether or not, the exclusion of seed and seedling predators, could improve the probability and feasibility of regeneration and restoration of ecosystems.

As a result of logging, forest fires and the unchecked presence of livestock, central Chile has sustained an ongoing degradation of its ecosystems over the past centuries (Aronson et al. 1998, Armesto et al. 2010). Currently, vegetation in this region is arranged as a mosaic of forest patches, shrub patches and open areas (Fuentes et al. 1984). In central Chile, studies of woody species emphasize the significant influence of rainfall on regeneration density and diversity (Gutiérrez et al.

2000, Jaksic 2001, Holmgren & Scheffer 2001, Jaksic 2001, Holmgren et al. 2006, Becerra et al. 2016). Also, a positive effect of woody cover on the regeneration of woody species has also been reported (Bustamante 1991, Armesto & Pickett 1985, Fuentes et al. 1986, Becerra & Montenegro 2013). Additionally, herbivory in this region has also been documented as an important limiting factor in the regeneration of woody species, although these studies have mostly focused on rabbits and cattle (Fuentes et al. 1983, Holmgren 2002, Morales et al. 2015), and there is less research and information with respect to seed predation. The herbivory on seedlings has been studied through planting experiments, rather than observations of herbivory on natural regeneration, or germination of plants *in situ*. Some reports indicate that there is in fact, seed predation on certain species in central Chile (Bustamante & Simonetti 2000, Henríquez & Simonetti 2001, Celis et al. 2004, Morales et al. 2015). Nevertheless, the level or the extent of seed predation is unknown. Several studies report significant levels of plant herbivory in central Chile, mainly generated by rabbits (Simonetti et al. 1983, Fuentes et al. 1984, Morales et al. 2015). However, all these studies evaluating herbivory on seedlings used experimentally planted plants. There are no reports that analyze the impact of herbivory on natural regeneration or, on germinated plants *in situ.* Having this information would allow to understand the role and impact of herbivory for natural regeneration of sclerophyllous forest species.

In this research the role of vertebrate herbivores in the natural regeneration of woody species in central Chile, was evaluated. The degree to which herbivory levels were modulated by the specific type of vegetation patch was also analyzed. Vertebrate herbivore exclusion studies were developed in three types of vegetation patch (arboreal, shrubs, open). The studies were carried out in five localities in central Chile, in order to evaluate geographical generality of herbivory effects within the central region.

The analysis in Chapter 1 specifically evaluates the process of seed predation and the effect of herbivore exclusion on the removal of experimentally arranged seeds, from 6 native woody species, as well as those in the natural seed bank. It is expected that the exclusion of vertebrate herbivores will reduce seed predation and increase the seed bank. This should occur especially in patches of greater woody plant cover, where a higher prevalence and diversity of herbivores is expected. Chapter 2 studies the effect of herbivory on the regeneration of *in situ* germinated seedlings of woody species, under different vegetation patch conditions. Consequently, seeds of 6 woody species were sown experimentally inside and outside exclusions of vertebrate herbivores, and their recruitment was monitored. The natural regeneration was also evaluated in and out of exclusions. Exclusion analysis was carried out in different types of vegetation patches in 4 locations in central Chile. It is expected that recruitment from the experimental sowing, as well as the natural regeneration, will be greater inside rather than outside the exclusion area. These should mainly occur in patches with lower woody cover, assuming that the main herbivory will be generated by rabbits, such as that observed in previous studies, as regards the effect of these herbivores (Fuentes et al. 1983).

Chapter 2 of the study shows the final regeneration resulting from the effects of herbivory and vegetation patches in central Chile. In the sowing experiment of this study only two life cycle stages (i.e. seed and seedling) are analyzed. Thus, the results observed in this experiment show the effect of herbivores and vegetation patches, specifically on the post-dispersal stages, controlling the woody species composition as well as their initial seed availability. Hence, this may include the effect of seed predation, germination restrictions, seedling predation and restrictions on seedling survival. In contrast, the natural regeneration experiment evaluated in Chapter 2, may show results not only for seed and seedling stages, but also of previous life cycle phases such as reproduction and dispersal. For this reason, the natural regeneration experiment shows the net effect of herbivores and vegetation patches on natural regeneration. On the other hand, results shown in Chapter 1 only focus on one of the life cycle stages evaluated in Chapter 2, and are limited to the seed state, and its association to vegetation patches and herbivore effect. In reference to the seed predation experiment of the Chapter 1, the results exclusively show the post-dispersal stage of seed survival. In contrast, the experiment in which the seed bank is evaluated, indicates the net effect of vertebrate herbivores and vegetation patches on the quantity and diversity of seeds available for recruitment. Hence, this could be a consequence of both seed predation as well as reproduction and dispersal processes**.**

Results of this research will determine the relevance and the need to exclude vertebrate herbivores, namely birds and mammals, in order to improve natural regeneration, or planting and seeding of woody species in the context of restoration processes in central Chile.

Chapter 1

SEED PREDATION OF WOODY SPECIES VARIES AMONG DIFFERENT VEGETATION PATCHES IN THE MEDITERRANEAN-TYPE REGION OF CHILE

María José Correa-Solís[a,c], Pablo I. Becerra[a,b,]

[a] Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.

[b] Center of Applied Ecology and Sustainability, CAPES

[c] Laboratorio de Reproducción y Propagación de Especies Nativas, Ecorestaura Limitada

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Seed predation of woody species varies among different vegetation patches in the Mediterranean-type region of Chile

María José Correa-Solís[a,c], Pablo I. Becerra[a,b,*]

[a] Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.

[b] Center of Applied Ecology and Sustainability, CAPES

[c] Laboratorio de Reproducción y Propagación de Especies Nativas, Ecorestaura Limitada

*Corresponding author

Email addresses: pablobecerra@uc.cl (P.I. Becerra), mjose.correa.solis@gmail.com (M.J. Correa-Solís)

ABSTRACT

Seed predation reduces the quantity of seeds available to germinate, however, its magnitude may depend on the abundance of seed predators and therefore it may vary between habitats and geographically. This variability, and the impact of seed predation in the seed bank of plant communities are still little known. In this study we evaluated seed predation of woody species in different vegetation patch-types and localities from the Mediterranean-type region of Chile. In one experiment, we established 1 m^2 exclosures in forest, shrub and open patches in one Andean and a coastal locality. These exclosures excluded vertebrate seed predators such as birds, rodents and other mammals. We installed eight pots (one different species per pot), within and outside the exclosures with 20 seeds in every pot, and evaluated seed removal. In other experiment, we installed exclosures in the same vegetation patch-types in other three localities, and soil samples were collected in and outside of them to evaluate seed banks. Exclosures significantly reduced the number of seeds removed in the majority of species and patch types in both localities, with some exceptions in open sites. Exclosures significantly increased the number of seeds and species richness of the seed banks in some forest and shrub patches of four localities. The results suggest that seed predation is mainly produced by vertebrates and affects several woody species from central Chile, reducing the abundance and species richness of seeds available to germinate. This appears to be more common in forest and shrub patches, which were the patches where seed predators were more frequent. Our results highlight the role of seed predation for woody regeneration and suggest that this effect may be greater in microhabitats where seeds have greater probability to germinate and seedlings to survive in semiarid ecosystems.

Key words seed predation, seed banks, regeneration, sclerophyllous forest, central Chile

1. Introduction

Plant regeneration is critical for the structuring, succession and ecological restoration of species in communities (Grubb 1977; Crouzeilles et al. 2017). Different biotic and abiotic factors can hinder or facilitate the regeneration of woody plant species. Among these are granivory and seed predation, which for many years have been documented as important drivers for plant regeneration and vegetation dynamics since they strongly affect seed banks available for germination and recruitment. (Wenny 2000; Paine and Beck 2007; Vaz Ferreira et al. 2011; Larios et al. 2017). Plants can lose as much as 90% of their seeds owing to predation (Crawley 2000; Hulme 1998), which has led some to propose the seed stage as the most vulnerable in the life cycle of plants (Hanley et al. 2007). However, the impacts of seed predation on seed banks available to germinate at a community level are still little known.

Plant cover in degraded forest ecosystems is usually a mosaic of open sites where the forest has been lost and unrecovered, patches with some degree of recovery, generally dominated by pioneering shrubs, and well conserved or recovered patches that maintain a tree canopy (Fuentes et al. 1986; Schulz et al. 2010). Plant cover is considered an important factor affecting plant recruitment due to its influence on light, temperature, and soil moisture and nutrient conditions, which may be critical in the germination and seedling survival (García-Fayos and Verdú 1998; Valladares and Puignaire 1999; Jordano 2000; Maestre et al. 2003). For example, several works have documented that regeneration in semiarid climates occurs mainly under canopies in woody or shrub patches, because plants suffer less water stress in that type of microhabitat (Maestre et al. 2003; Brooker et al. 2008). However, the type of vegetation patch (woody, shrub, or open) can also influence plant recruitment through the level of seed predation (e.g. Díaz et al. 1999; Kelt et al. 2004), which has been less studied. Many animal species prefer microhabitats with determined levels of cover, because of which the type of vegetation patch can influence the frequency and abundance of potential seed predators. For example, it has been documented that several animal species consider woody patches as offering more protection against predation. Rodents, which are mainly granivores, have been described as having anti-predatory behavior in their preference for patches with greater cover as refuge (Simonetti 1989; Guiden and Orrock 2017; Wang et al. 2019). Additionally, some vegetation patches may concentrate food, such as seeds, and hence seed predators may use more frequently those patch types (Simonetti 1989; Morris and Davidson 2000). In general, sites with more granivores suffer more seed predation (Rey et al. 2002; Mezquida and Benkman 2014), and therefore seed predation probably has greater magnitude and occurs on a higher species diversity in patches that are more often used by granivores (Schnurr et al. 2004). However, to date the role and importance of the vegetation patch type on the level of seed predation has not been heavily studied (but see Kelt et al. 2004; Schnurr et al. 2004).

Direct seeding is an important means of reforestation and restoration of ecosystems, and in some situations, can be the only viable way to reintroduce species owing to the lower costs and greater facility to reforest less accessible areas (Woods and Elliott 2004; Doust et al. 2006; Grossnickle and Ivetic 2017). The magnitude of seed predation and the diversity of woody species that suffer this is important information for taking decisions with respect to the viability of restoring ecosystems through direct seeding.

Central Chile has a semiarid Mediterranean-type climate with vegetation that has degraded significantly owing to tree-cutting, fires and livestock farming (Aronson et al. 1998; Armesto et al. 2010). Vegetation in the region currently has a mosaic structure composed of woody, shrub and open patches (Fuentes et al. 1984). The regeneration of woody species in central Chile has been relatively well studied in recent decades. Several works have documented an important role of precipitation in the density and diversity of regeneration (Gutiérrez et al. 2000; Holmgren and Scheffer 200; Holmgren et al. 2006; Becerra et al. 2016), while other works have observed a strong positive effect of woody cover on the regeneration of woody species in this region (Armesto and Pickett 1985; Fuentes et al. 1984, 1986; Becerra and Montenegro 2013). Seedling herbivory, mainly by rabbits and cattle, has also been reported as an important factor limiting the regeneration of woody species in this region (Fuentes et al. 1983; Holmgren 2002; Morales et al. 2015). However, the role of seed predation has been less studied. Some studies have documented that seed predation affects some few woody species in central Chile (Bustamante and Vásquez 1995; Bustamante and Simonetti 2000; Henríquez and Simonetti 2001; Celis et al. 2004; Morales et al. 2015). Other studies have shown that in the arid region of Chile, such as in other South American localities, birds and small mammals would be the main seed predators, and instead, ants do not seem predate importantly on seeds (Kelt et al. 2004). Also, comparisons between vegetation patches suggest that predation of the species native to central Chile, *Cryptocarya alba*, is greater in open scrub than in forest interiors (Bustamante and Vásquez 1995), but in general, seed predation by small mammals and birds is greater in more covered vegetation patches (Kelt et al. 2004). However, it is not known whether seed predation and the role of vertebrate vs smaller seed predators are widespread among different woody species in this region, and if seed predation has impact on seed banks at a community level. The role of the vegetation patch type in seed predation also remains unclear.

In this study, we evaluated seed predation of different woody species typical of central Chile and the role of vertebrate herbivores (birds and mammals together) in seed predation. We also examined whether the type of vegetation patch influences the level of predation. We first conducted a seed removal experiment using different species and three vegetation patch types in two localities in central Chile to compare the levels of removal within and outside of vertebrate exclosures. In addition, we evaluated whether the exclusion of vertebrate seed predators for two years increases the seed bank of woody species in different types of vegetation patch in 5 localities in central Chile. We expected to find, first, that seed removal occurs in all species, second, that there is less seed removal and greater abundance and species diversity in the seed bank within vertebrate exclosures than outside them, and third, that the effect of the exclosures is more significant in forest and shrub patches than in open sites that are less used by vertebrates such as small mammals and birds.

2. Method

2.1. Study area

The study was conducted in central Chile, in areas of scrubland and sclerophyllous forest. The region is characterized by a Mediterranean-type climate, with cold rainy winters and hot dry summers (Luebert and Pliscoff 2006). Since 2010, the region has been experiencing deficits in precipitation of up to 40% (Garreaud et al. 2020).

The study included five localities in total: San Carlos de Apoquindo Natural Park (SC) (1042 m.a.s.l.), Lago Peñuelas Forest Reserve (LP) (374 m.a.s.l.), Cascadas de las Ánimas (CA) (1082 m.a.s.l.), San Vicente (SV) (175 m.a.s.l.), Río de los Cipreses National Reserve (RC) (1020 m.a.s.l.) (Fig. 1). The localities are similar in the composition and structure of vegetation, which take the form of a mosaic of forest, shrub and open patches (Gajardo 1994). The predominant tree species of the forest patches are *Lithrea caustica, Quillaja saponaria, Cryptocarya alba,* and *Peumus boldus*. The predominant species in the shrub patches are *Baccharis paniculata*, *B. linearis*, *Colliguaja odorifera*, *Retanilla trinervia,* and *Proustia cuneifolia*.

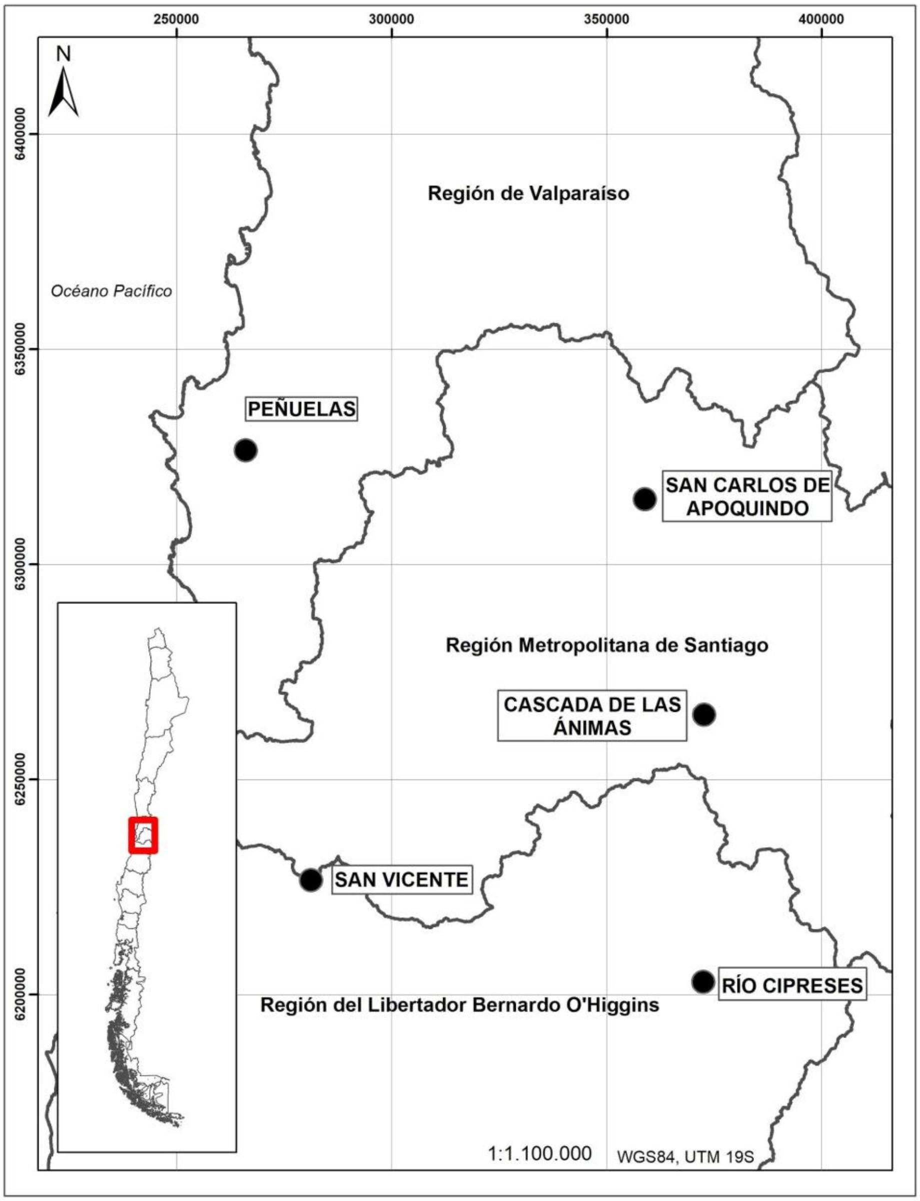

Fig. 1. Geographic locations of the studied localities.

2.2. Seed removal experiment

Eight woody species (*Acacia caven, Cryptocarya alba, Quillaja saponaria, Lithrea caustica, Maytenus boaria, Peumus boldus, Schinus latifolius*, and *Senna candolleana*) were employed in this experiment to evaluate seed predation. All these species produce seeds that are dispersed between January and May. Seeds of all the species were collected in the localities SC and LP between January and April 2017. Only completely mature fruits were collected. The pulp and cover were removed from around the seeds of species with fleshy fruits (*P. boldus, C. alba, M. boaria*) and species with drupes with less fleshy covers (*L. caustica, S. latifolius*) to assure that seeds were not removed by potential seed dispersers. The seeds were removed from the fruit in species with dry fruit in pods (*A. caven* and *S. candolleana*), and *Q. saponaria* (winged seeds in capsules). Only seeds with no evident damage by insects were employed.

This experiment was conducted at two locations (LP and SC), at each of which a surface area of approximately five hectares was used, in which 10 forest patches, 10 shrub patches, and 10 open sites were selected. The forest patches were > 6 m high, with surface areas of 40-200 m^2, while the shrub patches were 2-4 m high, with surface areas of 30-100 m^2. In general, the open sites always featured an herbaceous strata of < 0.5 m, with surface areas of 60-400 m^2. Exclosures measuring 1 by 1 meter and 0.5 m high were established in all 60 patches in 2015. The exclosures consisted of wooden frames supporting wire meshes with an opening diameter of 1.2 cm. Non-exclusion areas of the same dimensions were marked next to the exclosures. In June 2017, eight plastic pots, 15 cm in diameter and 10 cm deep were placed 20 cm apart in every exclosures and neighboring non-exclusion area. Twenty seeds were placed in all the pots, with one species per pot. After placing the seeds, the pots were monitored for 18 days to determine the number of seeds that remained or were removed.

2.3. Seed bank experiment

This study was conducted at the five localities in each of which 10 forest patches, 10 shrub patches and 10 open sites were selected. Vertebrate exclosures were installed in all the patches in the winter of 2015, similar to what was described in the previous experiment. Soil samples 20 by 20 cm by 5 cm deep (including a leaf litter layer when present) were collected from within and outside of all the exclosures (0.5 m from the exclosures) after the end of the seed dispersion period in June 2017. In all, 300 samples were taken. The seeds in the samples were counted and identified in the laboratory.

2.4. Sampling of potential seed predators

The presence of possible vertebrate seed predators was assessed in the localities where the seed removal experiment was conducted (SC and LP). Animals 1.5 cm in diameter and larger were excluded from the exclosures. This probably did not prevent the entry of reptiles, insects or smaller organisms. Therefore, the types of vertebrates excluded that may have caused the observed differences between exclusion and non-exclusion treatments were birds and mammals like rodents, cattle, goats, horses, and others. The samplings described below were conducted to establish the possible seed predator species and their frequency in using each studied type of patch.

Birds were sampled based on counting points that consisted of registering all the individuals observed by direct sighting or recognize by vocalizations in an area of approximately 100 m^2 (de la Maza and Bonacic, 2013). Playback of birdsong were used to encourage vocalizations. The counting points were located concentrically in the patches with exclusion areas, in order to associate specific counting points with a particular patch-type. At each sampling point, we waited two minutes before beginning to register data, at which moment we began registering all species and the number of individuals that appeared in the counting points during a ten-minute period. The counting point samplings were all carried out between approximately 8:00 and 11:00 AM over three consecutive days at each locality (SC: July 27 to 29, 2017, and LP: August 1 to 3, 2017). Three counting point samplings were made every day in each patch type, for 9 counts in all. This method permitted studying the different compositions among habitats or patch types, and the level of use of habitats by every species, although it was not necessarily possible to establish a density value per species (Ralph *et al.* 1993, de la Maza and Bonacic 2013).

Mammals were sampled by two techniques, cameras-traps and Sherman traps. Six cameras-traps were installed in the vegetation patches (two per type of patch) and maintained for eight consecutive days, beginning July 20 in SC, and July 29 in LP. The cameras were aimed at the areas where the pots had been placed. The Sherman traps were installed in SC and LP on July 22 and 29, respectively. Thirty traps were installed at every site, ten in each patch type where pots with seeds had been installed. The traps were primed with oats and conditioned with pieces of cotton. The traps were checked daily in the morning (8:00 AM) for four days. When small mammals were found in the traps, the species was identified and registered and the animal was released. The traps were removed after the fourth night.

2.5. Statistical analysis

In the seed removal experiment we determined the number of seeds removed per patch and species (hereinafter "population analysis"), the total number of seeds removed (combining all species) and the number of species (richness) that suffered seed removal from among the eight studied species (hereinafter "community analysis"). The total number of seeds (all species combined) and the number species (richness) in the seed bank test samples were quantified (only "community analysis"). A population analysis was not carried out in this case (by species) owing to the low number and frequency of appearance of seeds of the same species among all the analyzed samples of seed banks.

The data from the two experiments were analyzed by generalized linear models (GLM). The statistical analysis at the population level considered the vertebrate exclusion, the vegetation patch type and the species as fixed factors, including the statistical interaction among these factors. In the community-level analysis for the seed removal experiment (total abundance and species richness), the vertebrate exclusion and vegetation patches were considered fixed factors. Their interaction was also analyzed statistically. The community-level analysis for the seed bank was applied separately to each vegetation patch type. In this case, we did not consider the patch type as a factor because the difference in seed banks among patches could have been due to causes other than seed predation, such as seed dispersion. All the analyses were applied separately for the different localities in order to examine whether these effects are repeated in the different geographical locations in the studied region. According to the type of variables (counts), we used Poisson distribution of data and Log link function in every analyses, with the program R 3.3.1 and the “stats” package (Studio Team, 2016).

3. Results

3.1. Seed removal experiment

The number of seeds removed in the population analysis varied significantly among the exclusion treatments, vegetation patches and plant species in the two localities (Table 1). The interaction among these factors was also significant, indicating that the effects of the exclusion treatments, patch type and species are interdependent. Analyzing the effects of these factors separately, significantly more seeds of all the species were removed from outside the exclosures than in them in all the forest and shrub patches in both localities (Fig. 2). In contrast, the removal of seeds of the species *A. caven* was not significantly affected by exclosures in open sites in LP (Fig. 2). Likewise, seeds of the species *S. latifolius* and *S. candolleana* were not affected by the exclosures in open sites in the locality of SC (Fig. 2). Seed removal (combining in and outside the exclosures) by the patch type was greater in forest patches than in shrub patches or open sites in LP, while there was no significant difference among patch types in the locality of SC (Table 1S). Seed removal from outside the exclosures (seeds available to any type of seed predator) in both localities was significantly higher in forest and shrub patches than in open sites (Table 1S). Making this comparison (only outside the exclosures) separately by species in the locality of LP, seed removal was only significantly greater in forest patches than in open and shrub patches in four species (*P. boldus, A. caven*, *M. boaria* and *C. alba*), while in two species (*S. candolleana* and *S. latifolius*), seed predation was significantly greater in open sites and forest patches than in shrub patches (Fig. 2). In the locality of SC, seed removal of three species (*L. caustica, M. boaria, S. latifolius*) was significantly higher in forest and shrub patches than in open sites, while in two species (*P. boldus* and *S. candolleana*) seed removal was greater in forest patches than in the other two patch types (Fig. 2). In one species (*A. caven*), seed removal was greater in shrub patches than in the other two types, and in another species (*C. alba*), seed removal was greater in open sites than in forest or shrub patches. Finally, there was no difference in the level of seed removal of the species *Q. saponaria* among the different patch types in both localities (Fig. 2)

The analysis at a community-level showed that the total abundance of removed seeds varied significantly between exclusion treatments and among patch types in both localities, and that the interaction between these two factors was significant (Table 2). In contrast, the species richness of removed seeds varied significantly between exclusion treatments but not among the patch types, and the interaction between the two factors was also significant in both localities (Table 2). Although total abundance and species richness of removed seeds were greater outside than in exclosures in all patch types (Fig. 3), differences among patch types were not similar in the two exclusion treatments (Fig. 3). Considering only the treatment without exclusion (where seeds were available to all types of seed predators), the total abundance of removed seeds was significantly greater in forest patches than in the other two patch types in LP, while in SC seed removal in forest patches was only greater than in open sites (Fig. 3). Outside exclosures, the species richness of removed seed was greater in forest patches than in open sites in LP, while it did not vary among patch types in SC (Fig. 3).

Table 1 Statistical results (GLM) for the abundance of the species of removed seeds at the two localities over the experimental period. Numbers in bold indicate statistically significant differences ($P < 0.05$).

Source of variation	San Carlos		Peñuelas	
	Chi-square	P	Chi-square	P
Patch	8.253	**0.016**	87.618	**<0.001**
Exclusion	782.569	**<0.001**	1461.134	**<0.001**
Species	498.386	**<0.001**	343.858	**<0.001**
Patch * Exclusion	35.135	**<0.001**	59.580	**<0.001**
Patch * Species	126.656	**<0.001**	74.394	**<0.001**
Exclusion * Species	59.969	**<0.001**	22.224	**0.001**
Patch * Exclusion * Species	37.119	**<0.001**	20.238	**0.001**

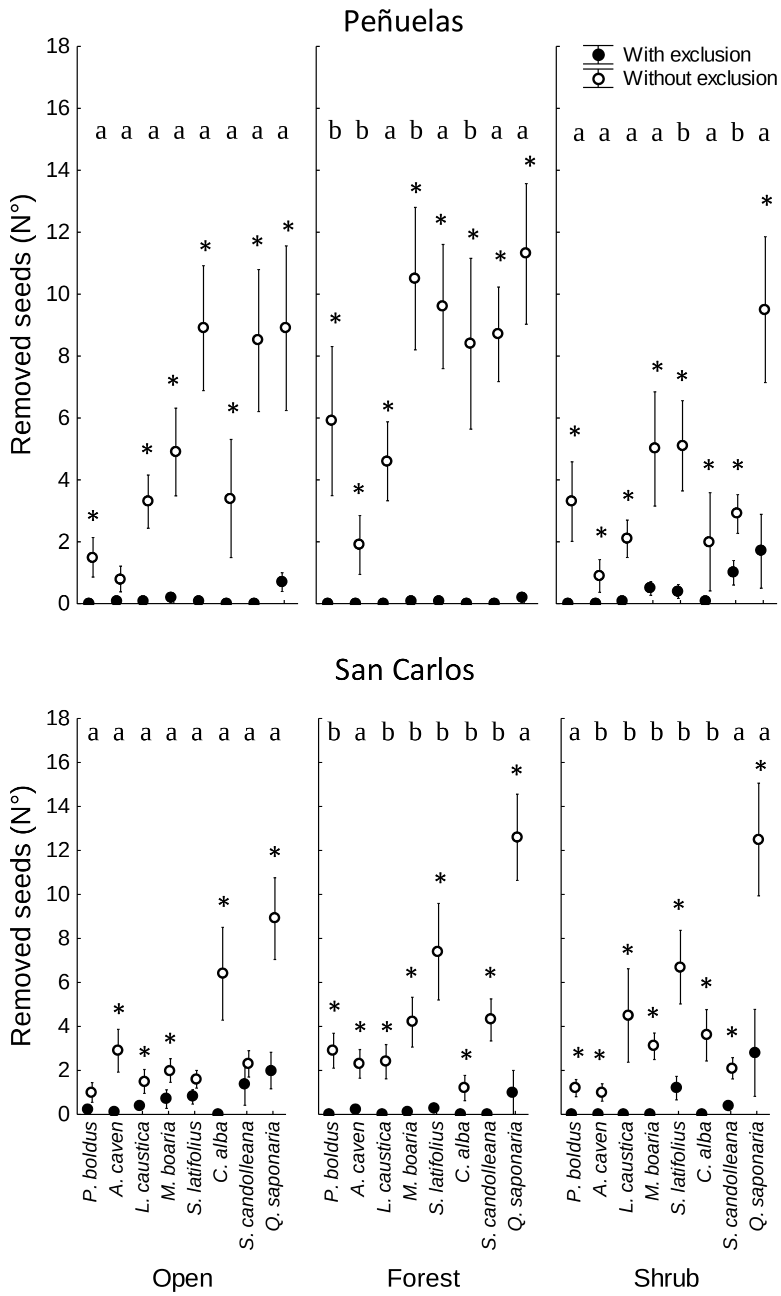

Fig. 2. Number of seeds of every woody species removed during the experimental period from all the vegetation patches. * indicates significant differences (GLM, P < 0.05) in seed removal between areas with and without exclusion. Different letters indicate significant differences (GLM, P < 0.05) among vegetation patches separately per each species and locality in the treatment without exclusion.

Table 2 Statistical results (GLM) for total abundance (combining all the species) and species richness of the seeds removed in the two studied localities over the experimental period. Numbers in bold indicate statistically significant values (P < 0.05).

Source of variation	Total abundance of removed seeds				Species richness of removed seeds			
	Peñuelas		San Carlos		Peñuelas		San Carlos	
	Chi-square	P	Chi-square	P	Chi-square	P	Chi-square	P
Patch	94.822	**<0.001**	9.240	**0.009**	0.355	0.837	2.362	0.306
Exclusion	1525.116	**<0.001**	1005.624	**<0.001**	120.320	**<0.001**	99.327	**<0.001**
Patch * Exclusion	35.820	**<0.001**	30.480	**<0.001**	10.473	**0.005**	12.101	**0.002**

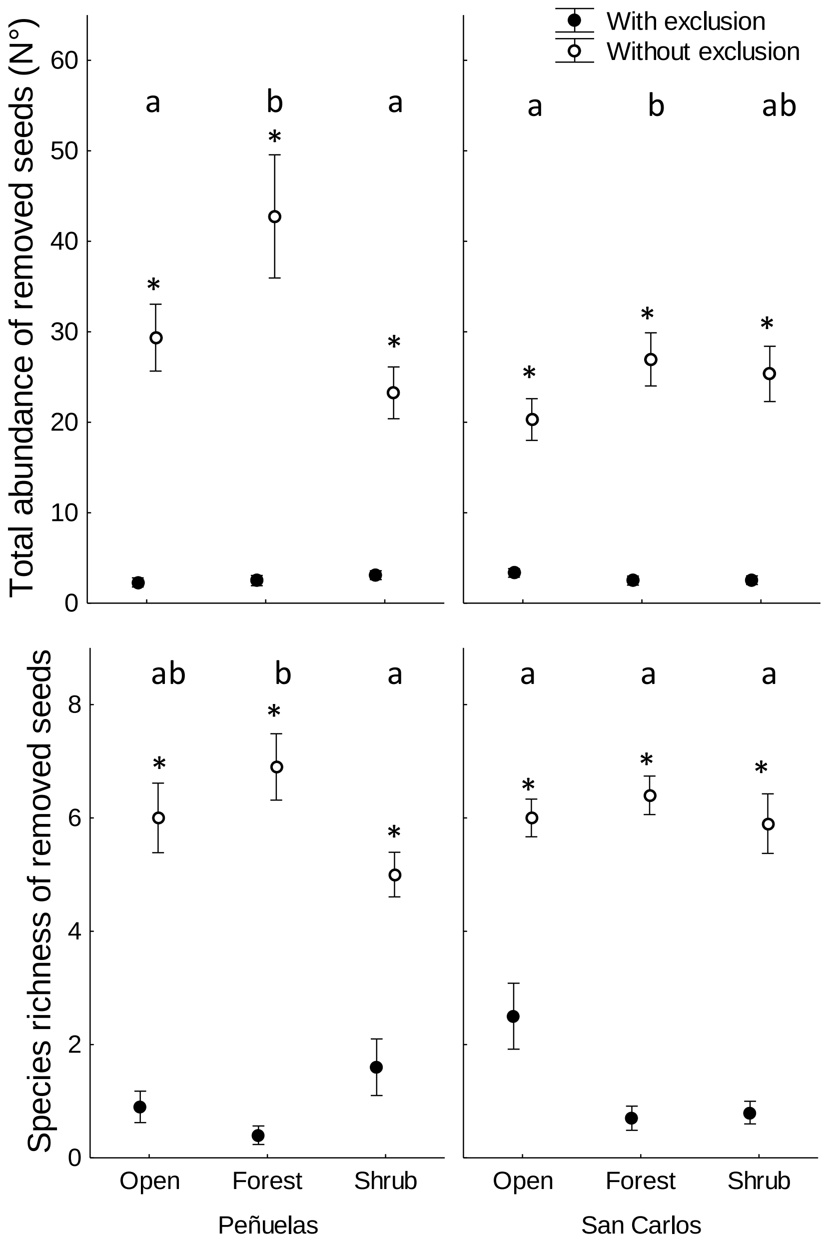

Fig. 3. Abundance (number of seeds) and species richness in seeds removed over the experimental period in and outside the exclusion areas according to the patch type and locality. * indicates significant differences (GLM, $P < 0.05$) in seed removal between treatments with and without exclusion for each type of patch and locality. Different letters indicate significant differences (GLM, $P < 0.05$) between vegetation patches per each locality in the treatment without exclusion.

3.2. Seed bank experiment

Combining all vegetation patch types, the abundance of the seed bank was significantly greater in the exclosures than outside them in all localities, except CA (Table 3, Fig. 4). However, more variability was observed when the effect of exclusion on seed banks was analyzed separately per patch type. In RC, seed bank abundance was significantly greater in the exclosures than outside them in the forest and shrub patches, while this was only the case in LP in forest patches, and in SC only in shrub patches (Fig. 4). Although there was a significant effect of exclusion in SV, this effect is diluted when analyzed by patch type and as a consequence, in no type of patch did exclusion affect the abundance of the seed bank (Fig. 4).

With respect to the species richness of the seed bank, when combining all types of vegetation patches, it was significantly greater within the exclosures than outside them only in the locality of LP and CA (Table 3, Fig. 4). When the analysis was made separately by vegetation patch type, only in forest patches of LP and in shrub patches of CA, the species richness of the seed bank was significantly higher within the exclosures than outside them (Fig. 4).

Table 3 Statistical results (GLM) for the exclusion effect (combining all types of vegetation patches) on total seed abundance (combining all species) and species richness observed in the seed bank of the studied localities. Numbers in bold indicate statistically significant values (P < 0.05).

Locality	Total abundance		Species richness	
	Chi-square	P	Chi-square	P
Cascadas (CA)	0.441	0.506	1.533	0.215
Río Cipreses (RC)	220.211	**<0.001**	0.021	0.884
Lago Peñuelas (LP)	88.795	**<0.001**	11.508	**<0.001**
San Carlos (SC)	34.148	**<0.001**	1.333	0.248
San Vicente (SV)	56.089	**<0.001**	0.083	0.772

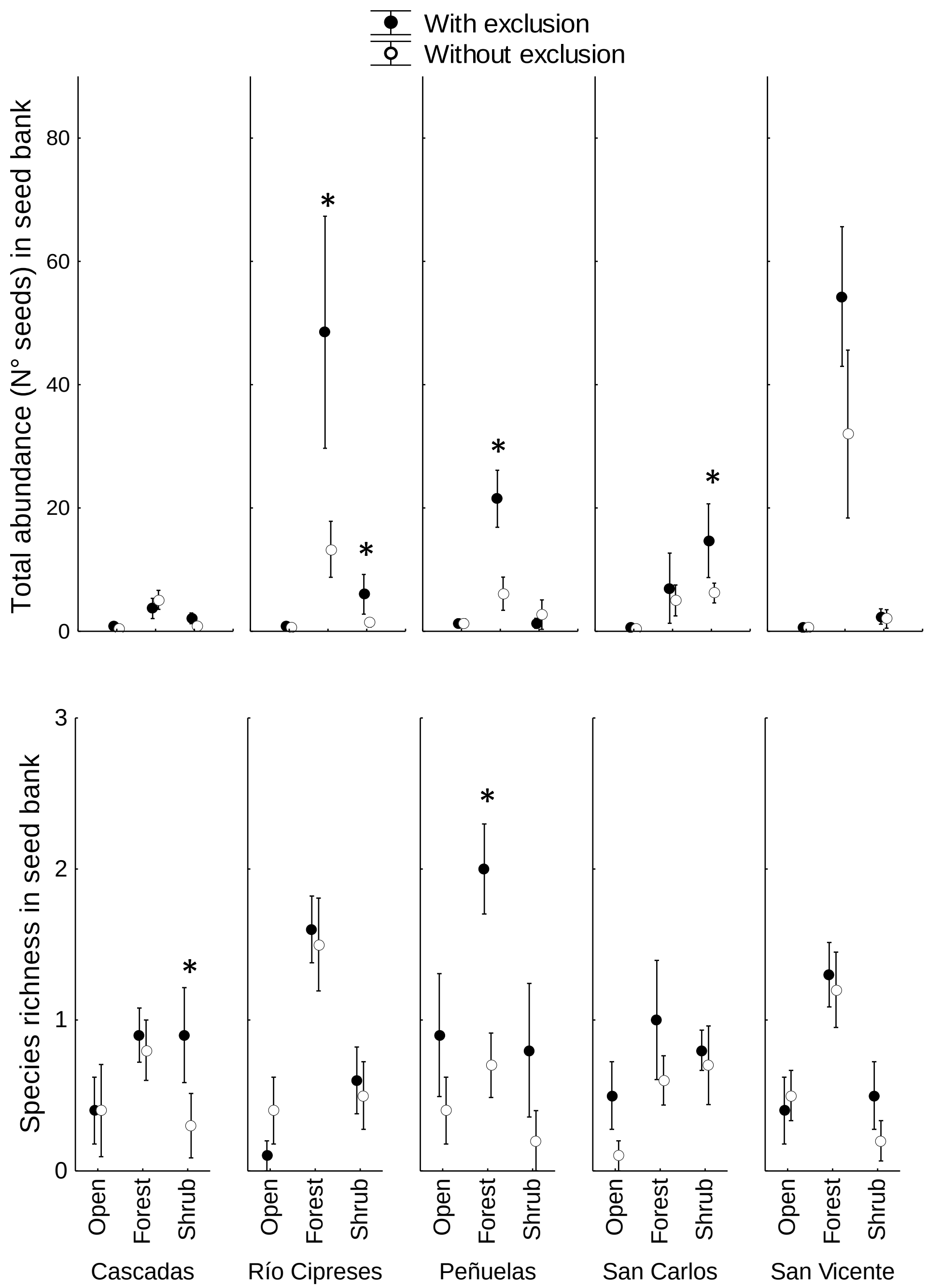

Fig. 4. Abundance (total number of seeds) and species richness of the woody species in seed banks of the studied patches and localities. * indicates significant differences (GLM, P < 0.05) between exclusion treatments per each patch type and locality.

3.3. Community of potential seed predators

In total, 19 vertebrate species of potential seed predators were observed, 15 bird species and four mammalian species. This total includes only species that the literature indicates contain seeds in their diets or that were observed consuming seeds during our sampling. In LP, 13 bird and two mammalian species were observed, while in San Carlos, 15 bird and two mammalian species were observed (Table 4). Species richness was clearly greater and there was more abundance of individuals in forest than in shrub patches, and in shrub than in open patches in both localities (Table 4).

Table 4 Community of potential seed predators observed at the two localities in the study on seed removal. The diets of all the species are documented or were observed (F=fruit, H=fungi, I= insects, N=nectar, S=seeds, V=vegetables, O=omnivore species), and abundance (number of individuals) registered at each type of patch (*introduced species).

Species	Diet	Lago Peñuelas			San Carlos		
		Open Site	Shrub Patch	Forest Patch	Open Site	Shrub Patch	Forest Patch
BIRDS							
Anairetes parulus	I, S	0	2	10	0	2	3
Callipepla californica *	S, I	0	32	36	0	32	45
Columbina picui	S	0	0	0	0	0	2
Curaeus curaeus	S, I, F	10	11	38	3	25	37
Diuca diuca	S, I	1	8	13	1	1	8
Leistes loyca	I, F, S	25	17	13	18	11	8
Mimus thenca	I, F, S, N	5	5	12	3	5	11
Molothrus bonariensis	S, I	0	0	0	17	16	23
Nothoprocta perdicaria	S, I	0	1	2	0	0	2
Phrygilus fruticeti	S, I, F	0	8	21	0	8	11
Phrygilus gayi	S, I	0	1	2	0	0	1
Sicalis luteola	S	0	16	18	0	17	23
Spinus barbata	S	0	3	4	0	3	5
Zenaida auriculata	S	8	10	21	6	8	20
Zonotrichia capensis	I, F, S	0	9	16	2	4	11
MAMMALS							
Abrothrix olivaceus	V,S,H	0	0	5	0	0	0
Lycalopex culpaeus	O	1	5	17	1	0	0
Thylamys elegans	O	0	0	0	0	0	2
Rattus norvegicus *	O	0	0	2	0	0	3
Species richness		6	14	16	9	12	17
Total abundance		56	142	230	51	132	215

4. Discussion

Our results suggest that seed predation of different woody species occurs, and that among the majority of species it occurs in different types of vegetation patches and different geographic localities, indicating that the phenomenon is generalized throughout central Chile. These results support the hypothesis that seed predation significantly reduces the availability of seeds for germination and recruitment (Wenny 2000; Paine and Beck 2007; Vaz Ferreira et al. 2011; Larios et al. 2017). Similar results, although based on very few woody species (*C. alba, B. miersii*) have been reported by other works on central Chile (Bustamante and Vásquez 1995; Bustamante and Simonetti 2000; Henríquez and Simonetti 2001; Celis et al. 2004; Morales et al. 2015) and on temperate forests in southern Chile (Díaz et al. 1999; Donoso et al. 2003). The fact that most predation occurred outside the vertebrate exclusions suggests that the main seed predators in this region are vertebrates larger than 1.2 cm in diameter (rodents and birds). Nevertheless, seed predation also occurred in the exclosures, although at a very low magnitude, which suggests that seeds in this region may also be preyed on by small predators (e.g. reptiles and insects), although this predation probably would be marginal for the seed bank.

Our results also suggest that the effect of seed predators like birds and rodents occur among the majority of species and in most vegetation patches and localities, and that the effect may only be insignificant in open areas in some few species (e.g. *A. caven, S. latifolius, S. candolleana* in our study). The results also suggest that the general level of seed predation of the majority of species (in the non-exclusion areas) is greater in forest patches than in shrub patches or open sites, although this depends on the locality. There are also species where the level of predation does not vary among patches, or is even higher in open sites than in forest or shrub patches. Therefore, the effect of patch type on the level of seed predation by species depends strongly on the species and locality.

At the community level, seed predation of woody species by vertebrates reduced the number of seeds and the species richness available for germination and recruitment in all the vegetation patches in the seed removal experiment. In the seed bank experiment, the effect of predators was only observed in forest and shrub patches, and not in all the studied localities. The observed differences could be the result of the different natures of the two experiments. Both experiments excluded the same types of seed predators, and the locations used in the removal experiment were also used in the seed bank experiment. However, in the seed removal experiment we used open pots from which seeds could easily be seen by predators. In contrast, seeds in the seed bank experiment (coming from natural dispersal) are located between or under an herbaceous layer, and sometimes partially buried under leaf litter, which may have reduced the probability of seeds being detected by predators. Furthermore, in localities where seed removal was not evaluated (and potential seed predators either), there may have been different species or differences in the abundance of seed predators, both those that were excluded and ones that were not, such as fungi, invertebrates, and reptiles.

At the community level, in terms of total abundance of seeds removed from outside the exclosures, the magnitude of seed predation was higher in forest patches than in open sites, and the contrast with the shrub patches depended on the locality. Instead, in terms of species richness, there were no differences between forest patches and open sites, and only in the locality of LP it was greater in forest than in shrub patches. Therefore, the vegetation patch effectively affected the magnitude of the seed predation, but this strongly depended on the analyzed variable and the locality.

The characteristics of habitats or microhabitats influence animal behavior, the presence and abundance of determined species and community composition (Morris and Davidson 2000; Mabry and Stamps 2008). Thus, plant-animal interactions such as seed predation, may depend on diverse habitat variables, for example woody cover (Brown and Ojeda 1987; Chase 1998; Rey et al. 2002; Orrock et al. 2010; Silliman et al. 2013; Mezquida and Benkman 2014). Our results are consistent with these observations. More species richness and individual frequency of seed predators were observed in forest and shrub patches than in open sites in both localities where we evaluated seed predators, suggesting a common pattern in central Chile. This is consistent with the higher level of seed removal observed in woody patches than in open sites in both localities, at least in terms of total abundance predated seeds. It is also consistent with the observed effects of excluding predators on seed removal and seed banks. Although seed removal at the community level was significantly reduced within exclusions in all patch types, we observed only in open sites a lack of significant effects of predator exclusion on seed removal in some species. Similarly, the only vegetation patch type where we did not observe a significant reduction in the seed bank due to exclusion was in open sites. This suggests that seed predation in this region is less important and less common in sites without woody cover, probably because seed predators make less use of open sites. In this region, this pattern of microhabitat use by fauna could be due to greater protection and refuge against predators offered by woody patches and/or greater concentration of food (seeds) in woody patches owing to more seed dispersion under tree canopies (Simonetti 1989; Lazo et al. 1990, Kelt et al. 2004). In fact, in our study, the seed banks in the exclusion areas were always greater in forest and shrub patches than in open ones. Jiménez and Armesto (1992) documented the same earlier. Other works have observed that seed predation is greater in large forest patches than in small ones or in the borders of large patches (Burkey 1993; Díaz et al. 1999). However, other studies have reported results that differ from ours. For example, Bustamante and Vásquez (1995) documented a higher rate of seed removal of *C. alba* (a species included in our study) in areas of open scrub than under the canopy of sclerophyllous forest. Interestingly, the only species in our study that had a higher seed predation in open sites than in forest and shrub patches was *C. alba*, at least in one of the localities. Other studies have also observed more seed predation in small forest fragments than in large ones, or on the edge of forests than in their interior (Santos and Tellería 1994, 1997; Bresciano et al. 1999; Donoso et al. 2003). These different patterns are probably related to how seed predators use the different types of habitats in different geographic localities.

Our results suggest that seed predation is a limiting factor for the regeneration of woody species in this region. However, for seed predation to limit regeneration, it is necessary that predation occurs in habitats or microhabitats where seeds have a relatively high probability of germinating and recruiting (Harper 1977). Woody species in central Chile are more likely to germinate and survive under a canopy in forest or shrub patches (Armesto and Pickett 1985; Fuentes et al. 1986; Becerra and Montenegro 2013), where we effectively found more seed predation. Therefore, our results suggest that seed predation may be limiting the regeneration of woody species in this region.

Direct seeding is an important way to reforestation and ecosystem restoration, and in some situations is the only viable method to reintroduce species owing to the lower costs and greater facility to reforest more inaccessible areas (Woods and Elliott 2004; Doust et al. 2006;

Grossnickle and Ivetic 2017). In our seed removal experiment, no more than 30% of seeds were removed over the 18-day monitoring period in any of the patches, although there was also no clear asymptote in the level of seed removal (Figure 1S). This suggests that seed removal rates will continue to increase and that the exclusion of predators would be important to reduce predation during seeding. However, the seeds placed in pots in our experiment were uncovered and visible to seed predators. In contrast, in manual seeding, seeds may be located under a layer of substrate, or with broadcast seeding, seeds may be located between the herbaceous layer and leaf litter, when present. Consequently, seeds would be less visible to predators, similar to what occurred with the seed bank in our study. In fact, the effect on seed banks of seed predator exclusion is minor and occurred in fewer locations and patches. This suggests that excluding seed predators during seeding may be less important when there is an herbaceous or leaf litter layer. Moreover, if sowing is buried, exclusion may not be necessary, especially if sowing is carried out with abundant quantities of seeds producing a mass effect. This requires additional research.

In conclusion, seed predation is effectively occurring among several species in central Chile and is reducing the number of seeds available to germinate and regenerate. Seed predation occurs in different types of vegetation patches, although it appears to be more common and of greater magnitude in forest patches than in open or shrub patches. However, this depends strongly on the species and locality, which makes seed predation highly complex and difficult to generalize.

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Supplementary Material

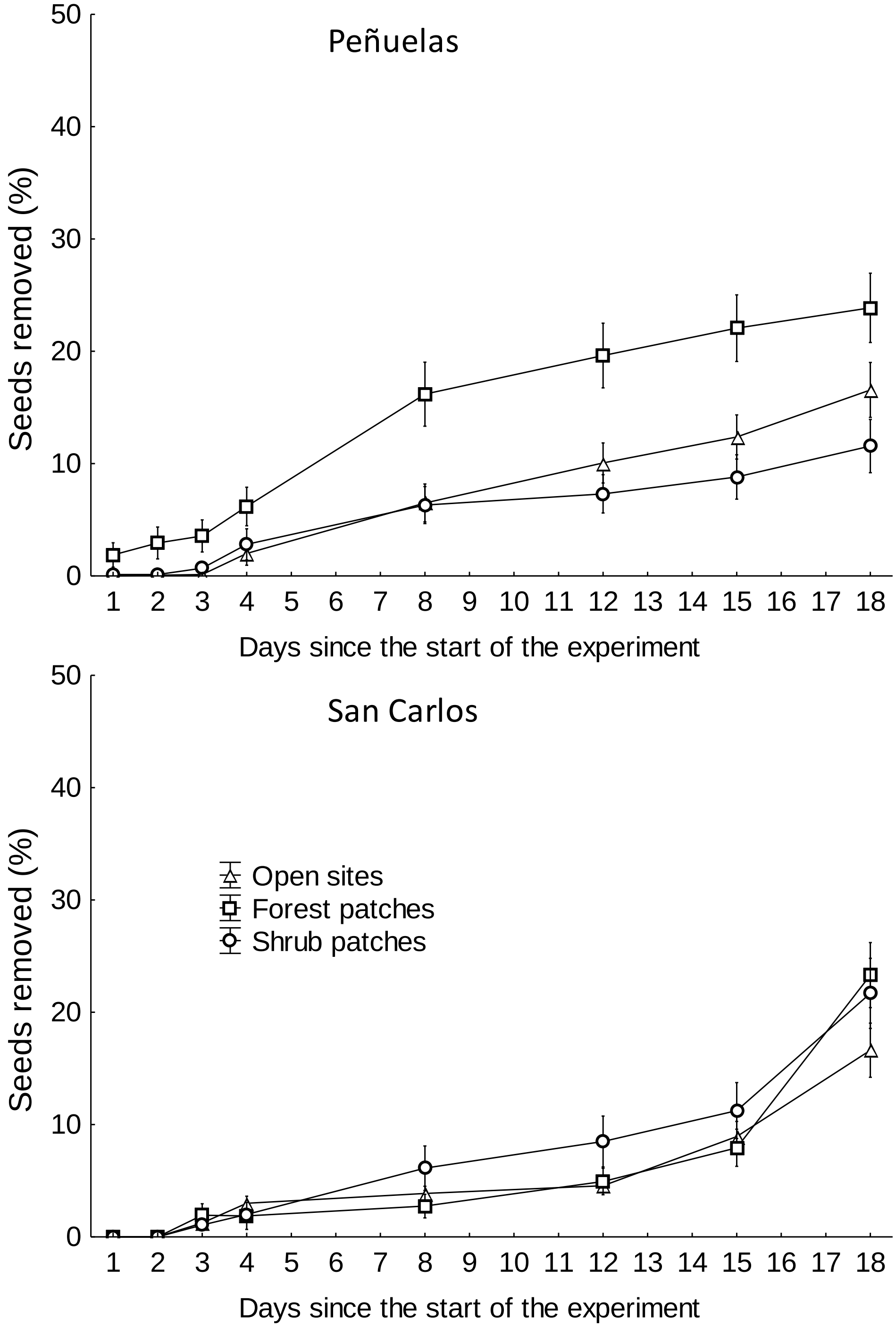

Figure 1S Seed removal (%) (considering only the treatments without exclosures) according to patch type in the two localities.

Table 1S Number of seeds removed (in the population analyses) for each vegetation patch type and exclusion treatment in the two studied localities. Statistical results (GLM, $P < 0.05$) for comparisons among patches and between exclusion treatments are also shown. In these cases, different lowercase letters indicate significant differences among patches (pooling both exclusion treatments), and between exclusions (pooling the three patch types). For the rest of comparisons, different uppercase letters indicate significant differences among patch types per each exclusion treatment separately, and lowercase letters indicate significant differences between exclusion treatments per a single vegetation patch type.

Patch	Exclusion treatment	N	Peñuelas			San Carlos		
			Mean	S.E.		Mean	S.E.	
Open		160	2.588	0.389	a	2.013	0.277	a
Forest		160	3.831	0.484	b	2.431	0.345	a
Shrub		160	2.163	0.320	a	2.444	0.370	a
	Without	240	5.496	0.399	a	4.108	0.327	a
	With	240	0.225	0.060	b	0.483	0.116	b
Open	Without	80	5.025	0.676	Aa	3.325	0.482	Aa
Open	With	80	0.150	0.051	Ab	0.700	0.183	Ab
Forest	Without	80	7.613	0.762	Ba	4.663	0.579	Ba
Forest	With	80	0.050	0.025	Ab	0.200	0.128	Bb
Scrub	Without	80	3.850	0.559	Ba	4.338	0.623	Ba
Scrub	With	80	0.475	0.168	Bb	0.550	0.267	Ab

Table 2S Species composition found in the seed bank per each exclusion treatment (E: exclosure, C: control), vegetation patch type (O: open, S: shrub, F: forest) and locality. Total number of seeds counted in the total number of samples (10) per patch type and locality is shown.

Species	Cascada de las ánimas						Río Cipreses						Lago Peñuelas						San Carlos						San Vicente					
	O		S		F		O		S		F		O		S		F		O		S		F		O		S		F	
	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C
Acacia caven	3	1	16	6					2	0	1	1	2	0	5	0	7	0	3	0	0	1	1	0			1	0	1	0
Aristotelia chilensis						4																								
Azara dentata																							0	3						
Baccharis paniculata									25	0											101	33	0	22						
Colliguaja odorifera																			2	0	5	2								
Cryptocarya alba	0	1	0	1							55	18	3	0			13	18	1	0										
Kageneckia oblonga					2	0																								
Lithraea caustica		2	2		21	15	0	3	5	9	385	66	4	1	0	2	1	0	0	5	39	20	9	8	4	3				
Maytenus boaria	2	0			2	0					0	1	3	1	3	0	1	7					1	0			3	0	0	1
Muehlenbeckia hastulata						1																								
Peumus boldus	1	0	2	0									1	6	1	1	27	0			0	1			1	3	2	4	514	284
Porlieria chilensis					1	0																								
Quillaja saponaria					6	21	1	2	5	5	44	47			4	0	165	36					58	17	1	1	1	0	25	33
Retanilla ephedra							7	1																						
Schinus polygamus																	1	0	1	0			1	0						
Trevoa trinervis	1	0											0	5							2	5					17	16	3	2
Species richness	4	3	3	2	5	4	2	3	4	2	4	5	5	4	4	2	7	3	4	1	4	6	5	4	3	3	5	2	4	4
Total abundance	7	4	20	7	32	41	8	6	37	14	485	133	13	1 3	13	3	215	61	7	5	147	62	70	50	6	7	24	20	543	320

Chapter 2

FACILITATION AND NOT HERBIVORY CONTROLS NATURAL REGENERATION OF WOODY SPECIES IN THE MEDITERRANEAN-CLIMATE REGION OF CENTRAL CHILE

María José Correa-Solís[1,3], Pablo I. Becerra[1,2]

[1] Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile.

[2] Center of Applied Ecology and Sustainability, CAPES.

[3] Laboratorio de Reproducción y Propagación de Especies Nativas, Ecorestaura Limitada.

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Facilitation and not herbivory controls natural regeneration of woody species in the Mediterranean-climate region of central Chile

María José Correa-Solís[1,3], Pablo I. Becerra[1,2]

[1] Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile

[2] Center of Applied Ecology and Sustainability, CAPES

[3] Laboratorio de Reproducción y Propagación de Especies Nativas, Ecorestaura Limitada

Correspondence

Pablo I. Becerra, Departamento de Ecosistemas y Medio Ambiente, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Av. Vicuña Mackenna 4860, Santiago, Chile. Center of Applied Ecology and Sustainability, CAPES

Email: pablobecerra@uc.cl

Abstract

Aim: Regeneration of woody species in semiarid ecosystems is often facilitated by the presence of woody patches. Herbivory can reduce the levels of regeneration, which may depend on the type of vegetation patch. However, it is still little known how the effects of these factors may interact. In this study, we evaluate the direct effects of vegetation patch types and herbivory, as well as their possible interaction, on the regeneration of woody species in central Chile.

Location: Five localities in central Chile distributed between 32° and 34° S.

Methods: We conducted an experiment that involved seeding six native woody species, with and without exclosures against vertebrate herbivores (birds, rodents, rabbits, cows, horses) in forest, shrub and open patches in two localities of this region. We also assessed natural regeneration with and without vertebrate herbivore exclusion in these types of vegetation patches in four localities. Results: The results of the seeding experiment indicate that the recruitment of the six studied species was facilitated by the presence of woody patches. The combined abundance of all the species and species richness were also higher under woody canopies than in open sites. Results from the natural regeneration experiment indicate that the woody patches facilitated regeneration among the different localities in the region. Vertebrate herbivores had a significant negative effect on the recruitment of some woody species in the region, mainly in forested and open sites, suggesting an interaction between vegetation patch type and herbivory. However, herbivory did not appear to have a significant effect on the levels of abundance or species richness of the natural regeneration.

Conclusions: The results suggest that regeneration of woody species in this region depend more on facilitation produced by woody patches than vertebrate herbivory, despite presence of different important herbivores (e.g. rabbits).
Key words: semiarid climates, facilitation, herbivory, vegetation patches, regeneration

INTRODUCTION

Regeneration is probably the most critical life-cycle stage for the structuring of plant communities, ecological succession and recovery of vegetation after perturbations (Harper 1977, García & Zamora 2003). Biotic interactions can strongly affect the abundance and diversity of regeneration (Grubb 1977). Degraded forest areas without woody vegetation are normally colonized by pioneering species that subsequently facilitate the regeneration of more advanced successional species, while the species of the final successional stages mainly regenerate in recovered forest patches or forest remnants (Armesto & Pickett 1985, Bonet 2004, Gómez-Aparicio 2009, Pugnaire et al. 2009). Successional recovery of forests in degraded areas as well as regeneration of late successional species in recovered forest patches can be hindered by native and exotic herbivores, either through seed and plant predation or the effects of trampling (De Steven 1991, Crawley 1992, Hanley 1998).

Vegetation in degraded areas in arid and semiarid climates is typically distributed as mosaics of woody, shrub and open patches (Fuentes et al. 1984, Aronson et al. 1998, Schulz et al. 2010). Numerous works have documented that plant-plant facilitation in these types of climates is especially important for the regeneration of vegetation. Consequently, regeneration largely occurs under the canopies of pioneering shrubs or trees, especially regeneration of intermediate or advanced successional species (Armesto & Pickett 1985, Callaway 1992, Pugnaire et al. 1996, Bonet 2004, Gómez-Aparicio et al. 2005, Maestre et al. 2009, Lloret & Granzow de la Cerda 2013, Caldeira et al. 2014). In contrast, open sites are recolonized almost exclusively by species adapted to high levels of solar radiation and drier and less fertile soils (Peterson & Picket 1990, Grime 1977). On the other hand, in many semiarid regions around the world, native and exotic herbivores with wide distribution (e.g. *Rattus spp, Oryctolagus cuniculus*, cattle and horses, among others) negatively affect the regeneration of woody species (Mills 1983, Zamora et al. 2001, Holmgren 2002, Figuerola & Green 2004, Young et al. 2013). However, the level of herbivory can depend on the successional or recovery state of vegetation, normally associated with the type of vegetation patch (woody, shrub, or open), mainly because of the preference of herbivores for microhabitats with particular levels of cover (Crawley 1992), or by indirect facilitation resulting from vegetation patches that reduce the effects of herbivory (Bertness & Callaway 1994). For example, it has been documented that patches or areas with more woody cover are preferred by herbivores because they offer more protection against predation on them (Guiden & Orrock 2017, Wang et al. 2019), because of which it is probable that there are more herbivores in patches with more woody vegetation (Fuentes et al. 1983, Díaz et al. 1999, Rey et al. 2002, Kelt et al. 2004, Schnurr et al. 2004). However, there is still little knowledge about the effects of vegetation patch types on the level of herbivory and with that, on the regeneration of woody species. Thus, interactive effects of vegetation patch-type and herbivory require further research.

Central Chile central has a semiarid Mediterranean-type climate. The vegetation in the region has been degrading for centuries as the result of logging and fires (Aronson et al. 1998, Armesto et al. 2010). Vegetation in the region has a mosaic structure of forest, shrub and open patches, the latter with only herbaceous cover. The patches represent different levels of vegetation recovery (Fuentes et al. 1984). The role of diverse ecological factors in the regeneration of woody species in central Chile has been studied in recent decades. For example, there is evidence that germination and seedling survival are higher in rainier localities or years (Gutiérrez et al. 2000; Holmgren, et al. 2006, Becerra et al. 2016, Velasco & Becerra 2020). Other works have documented the important positive effect of woody cover on the regeneration of woody species in this region (Armesto & Pickett 1985, Fuentes et al. 1984, 1986, Holmgren et al. 2000, Cuevas et al. 2013, Becerra & Montenegro 2013, Velasco & Becerra 2020). Although the impact of seed predation in central Chile has not been well studied (but see Bustamante & Simonetti 2000, Celis et al. 2004, Morales et al. 2015), seedling herbivory, mainly by rabbits, has been suggested as a very important factor limiting the regeneration of woody species in this region (Fuentes et al. 1983, 1984, 1986, Holmgren 2002, Morales et al. 2015). In other regions of the world, the European rabbit is also an important herbivore predating on herbs and woody plants (Bird et al. 2012, Tiver & Andrew 1997, Travers et al. 2017). However, all the studies performed on woody species have been based on experiments that compare the survival rates of planted seedlings protected from herbivores (in exclusion areas) to those of unprotected seedlings. Although this approach has allowed to establish the occurrence of herbivory on the studied plant species, it does not necessarily represent the importance of herbivory on natural regeneration. On the other hand, there is evidence that seed predators use more frequently forest patches and shrublands than open areas in central Chile (Kelt et al. 1994). Instead, in this region rabbits use more open patches and open shrublands than forest fragments (Jaksic & Soriguer 1981), which produces that herbivory by rabbits is greater in open habitats (Fuentes et al. 1983). Therefore, it is also unclear if the total effects of herbivory on natural regeneration vary among different vegetation patches in this region.

In this study, we evaluate the degree to which the natural regeneration of woody species in central Chile is affected by the type of vegetation patch and herbivory by vertebrates. As well, we examine whether the effect of herbivores depends on the type of vegetation patch. We expect to find that natural regeneration is positively affected by the woody cover, and that herbivores significantly reduce the level of regeneration. We also expect to find that herbivores have greater effect in open sites or sites with less woody cover than in forest patches, as has been observed in other studies with respect to the effect of rabbits (Fuentes et al. 1983). This last hypothesis assumes that rabbits are the main herbivores affecting the natural regeneration of woody species in this region. To evaluate these hypotheses, we first conducted a seeding experiment with and without exclusion of vertebrate herbivores and examined recruitment from germination *in situ* in different types of vegetation patches in two localities of central Chile. We also conducted an experiment in which we examined natural regeneration with and without exclusion of vertebrate herbivores in different types of vegetation patches at four localities in central Chile.

METHODS

Study sites

The study was conducted in central Chile in shrubland and sclerophyllous forest areas (Gajardo 1994). The climate is Mediterranean-type, with cold rainy winters and hot dry summers (Luebert & Pliscoff 2006). The area has been experiencing a 40% deficit in precipitation since 2010 (Garreaud *et al.* 2020). Five localities were included in the study, all located between 32° and 34° latitude south. The localities are San Carlos de Apoquindo Natural Reserve (SC) (1042 m.a.s.l.), Lago Peñuelas Forest Reserve (LP) (374 m.a.s.l.), Cascadas de las Ánimas (CA) (1082 m.a.s.l.), San Vicente (SV) (175 m.a.s.l.) and Río de los Cipreses National Reserve (RC) (1020 m.a.s.l.) (Figure 1). The vegetation of the localities is similar in composition and structure, taking the form of forest, shrub and open patches. Different localities were used in this study to determine the geographic generality of the effects of vegetation patch types and herbivory.

Seeding experiment

The seeding experiment employed six woody species (*Cryptocarya alba, Quillaja saponaria, Lithraea caustica, Maytenus boaria, Schinus latifolius* and *Senna candolleana*). The seeds of all these species were collected at two localities (SC and LP) between January and April 2017. Only completely mature seeds and/or fruit were selected. The pulp and cover were removed from around the seeds of species with fleshy fruits (*C. alba, M. boaria*) and from species with drupes with less fleshy covers (*L. caustica*, *S. latifolius*). The seeds were removed from the fruit of species with dry fruit in pods (*S. candolleana*), and capsules *Q. saponaria* (winged seeds). Seeds with apparent damage by insects were not used. The seeds were stored in a refrigerator for a month at 3 °C.

This experiment was conducted in the same two localities (LP and SC). An area of approximately five hectares was used in each locality, and within each area 10 forest patches, 10 shrub patches and 10 open stes were selected. The forest patches were all > 6 m high, with surface areas of 40 to 200 m^2, and generally dominated by *Lithrea caustica*, *Cryptocarya alba*, *Quillaja saponaria* and/or *Peumus boldus*. The shrub patches were 2 to 4 m high, with surface areas between 40 and 100 m^2, and dominated by the species *Baccharis linearis*, *B. paniculata*, *Colliguaja odorifera*, *Proustia cuneifolia*, and/or *Retanilla trinervia*. The open sites generally had an herbaceous strata < 0.5 m in height, with surface areas between 60 and 400 m^2. Exclosures were installed in the 60 patches of the two localities (30 per locality) in autumn, 2015. The exclosures, which were 1 x 1 m and 0.5 m high, were composed of four wooden supports covered on all sides and above by a wire mesh with openings 1.2 cm in diameter. A non-exclusion area with the same surface area was marked out beside each exclosure. In June 2017, 20 seeds of each of the six studied woody species were planted in the exclosures and the corresponding non-exclusion areas. The seeds were simply placed on the soil surface among leaf litter when it was present. Where there was no leaf litter, seeds were covered with a thin layer of soil to reduce the possibility of seeds being carried away by wind or water. Each species was sown in a surface area of approximately 20 x 20 cm, separated from the other species by at least 20 cm. A small flag was placed in the center of each sowing area to facilitate the subsequent detection of germinated seeds. Four months after sowing (November 2017), the numbers of surviving seedlings of each studied species were determined and recorded. In this way, the mortality of plants, especially in the non-exclusion areas, could have been due to seed predators, plant browsing, or trampling. Prior to sowing, weeds were removed in all experimental units to homogenize microenvironments among the experimental units.

Natural regeneration experiment

This study was carried out in the five localities, however, natural regeneration in SC was extremely poor, which impede us to do statistical analyses. Therefore, the study on natural regeneration was analyzed only in four localities (RC, CA, LP, and SV). In all these localities, ten sites for each of the three patch types (forest, shrub and open) were selected. Exclosures against vertebrate herbivores like those described above were set up in all the patches in autumn 2015. In autumn 2017, the number of plants < 0.5 high of all woody species were counted within a surface area of 0.5 x 1 in all the exclosures and neighboring (distance of < 0.5 m) non-exclusion areas. Germination of these species generally occurs in central Chile between August and November (spring). Consequently, the exclosures prevented herbivory during three recruitment periods (2015, 2016 and 2017).

Sampling of potential herbivores

We also evaluated the presence of possible herbivores that can affect regeneration by seed predation, plant browsing or trampling, at the localities where the seeding experiment was conducted (LP and SC). The exclosures affected animals larger than 1.2 cm in diameter. This did not exclude reptiles, insects or other small organisms. The excluded vertebrates, which could account for the differences observed between exclusion/non-exclusion treatments, were birds and mammals like rodents, rabbits, cattle, goats, horses and others.

Samplings were made to establish the possible species and frequency of use in each vegetation patch type. Birds were sampled using counting points, which consisted of registering all direct sightings and recognized vocalizations within an area of approximately 100 m^2 (de la Maza & Bonacic, 2013). A recording of bird vocalizations by playback was applied to encourage birds to vocalize. Each counting point was located concentrically in the patches where exclosures had been installed in the two localities. We waited two minutes at each sampling point and then began registering all the species and the number of individuals observed within the counting point for ten minutes. All the counting points were conducted between approximately 8:00 and 11:00 AM. The counting point samplings were carried out for three consecutive days at each locality (at SC on July 27, 28 and 29 2017, and at LP on August 1, 2 and 3 2017). Three counting points were carried out per day (at different patches) per patch type, so that there were nine samplings per patch type. This method assessed the specific composition among different types of habitats (in this case, vegetation patches), and the level of habitat use by different species, although it is not possible to establish the population density of every species (Ralph et al. 1993, de la Maza & Bonacic, 2013).

Mammals were sampled using two techniques, camera traps and Sherman traps. Six camera traps were installed in two of every patch type for eight consecutive days starting on July 20 in SC and on July 30 2017 in LP. The cameras were aimed at the areas where the seeds had been placed. The Sherman traps were installed in the localities SC and LP on July 25 and 30 2017, respectively. Thirty traps were installed at each locality in the same ten patches per patch type in which the seeding experiments were conducted. The traps were baited with rye and conditioned with a piece of cotton. The traps were checked daily in the morning (from 08:00 AM) for four days. When a small mammal was found in the trap it was identified by species and released. After the fourth night, the traps were removed.

Statistical analysis

The plants recruited among the 20 seeds sown in the seeding experiment were counted anad analyzed separately by species (hereinafter "population analysis"). The plants recruited (combining all species) among the 120 seeds sown per experimental unit were also counted, as well as the species richness in the seedling state (among the six species that were sown) (hereinafter "community analysis"). In the natural regeneration experiment, seedlings (combining all the observed species) and species richness in each experimental unit were quantified to perform a community analysis. A population analysis (separately by species) was not conducted owing to the low number of plants observed and the low frequency of finding the same species among the experimental units.

The data from the two experiments were analyzed using generalized linear modes (GLM), with the program R 3.3.1 and the Stats package (RStudio Team, 2016). The statistical analysis of data at the population level considered three fixed factors (patch type, exclusion and species). Two fixed factors (patch and exclusion) were used in the community analyses of the two experiments. All the analyses included evaluating the interaction among factors and *a posteriori* test among all the combinations of patches, herbivory treatments and species (in the population analysis), for which there was significant interaction. Another analysis considered in the design of the experiments was the evaluation of the geographic generality of the effects of the studied factors. For this reason, the experiments were repeated in different locations and the locations were analyzed separately. We preferred this approach than to include the locality as a fixed or random factor, firstly because it was not of interest to determine if different localities present different levels of regeneration, and secondly, we considered it important to determine exactly what happens in each locality to examine the level of geographic generality of the effects of patches and herbivory. Poisson data distribution and a logarithmic link function were used in all analyzes.

RESULTS

Seeding experiment

Recruitment from the seeding experiment varied significantly among the patch types and species in both localities in the analysis at the population level, while there were only significant differences between exclusion treatments at the locality SC (Table 1). There was significant interaction between patch type and species at the locality LP, while interaction among all three factors was observed at SC (Table 1). Comparing pairs of treatments, there was no significant effect of exclusion on recruitment with any species in the locality LP. Instead, in the locality SC, recruitment was significantly higher in exclosures than outside of them in open sites with the species, *S. latifolius*, in forest patches with the species *M. boaria* and *Q. saponaria*, and in shrub patches with the species *M. boaria* and *C. alba* (Figure 2). In the locality LP, the recruitment levels of the species *M. boaria* and *S. latifolius* in forest patches, and the species *S. latifolius* in shrub patches were significantly higher than in open sites (combining both exclusion treatments given that this factor did not interact significantly with species or patch type) (Figure 2). In the locality SC, the recruitment of four species (*L. caustica*, *M. boaria*, and *S. latifolius*, *Q. saponaria*) was higher in forest patches than in open sites, and the recruitment of four other species (*M. boaria, S. latifolius, C. alba,* and *Q. saponaria*) was significantly higher in shrub patches than in open sites, either in both exclusion treatments or in only one (Figure 2).

The community analysis of the seeding experiment showed that, at the locality LP, total abundance and species richness varied significantly among patch types, but were not affected by exclusion treatments or by the interaction between patch type and exclusion treatment (Table 2). Total abundance and recruited species richness in the locality LP were significantly higher in the forest and shrub patches than in open sites (combining the two exclusion treatments given that there were no differences between them in recruitment, and their interaction with the patch type was also not significant) (Figure 3). In contrast, at locality SC, abundance and species richness varied among patch types and between the two exclusion treatments, and the interaction between the two factors was significant (Table 2). Total abundance of seedlings and the richness of recruited species at this locality were significantly higher in the exclusions than outside of them in open and forest patches, while there were no significant differences between the exclusion treatments in the shrub patches (Figure 3). At SC, recruitment was significantly higher in forest patches than in open sites in both exclusion treatments, while only considering the areas without exclusion, recruitment was greater in the shrub than in the open patches (Figure 3).

Natural regeneration experiment

Total abundance and species richness of naturally recruited plants varied significantly among vegetation patch types in all the studied localities, but were not affected by the exclusion factor or by its interaction with the patch type (Table 3). However, a marginally significant interaction between patch type and exclusion was observed both on abundance and species richness ($P < 0.08$) in the locality of CA (Table 3). Total abundance and species richness were significantly higher in forest patches than in open sites in all the localities (Figure 4). As well, total abundance and species richness of the natural regeneration were significantly higher in shrub patches than in open sites in the RC locality, while in the LP locality only species richness was significantly higher in shrub than in open sites (Figure 4). Owing to the marginally significant interaction between patch type and exclusion treatment in the locality CA, we analyzed the differences between patches separately by the type of exclusion treatment (Figure 4). In this case, total abundance and species richness in this locality were significantly higher in forest patches than in open sites both in and outside the exclosures, while only in exclosures the abundance and species richness of naturally regenerated species was significantly greater in shrub than in open patches (Figure 4).

Community of potential seed predators

In total, 25 species of potential seed predators and/or consumers of plant organs were identified, 16 species of birds and 9 of mammals. Only species that the literature indicates consume plants (whether seeds or plant organs) or that were observed consuming plants during our sampling were included. At the locality LP, 14 bird species and eight mammal species were observed, while 16 bird and 6 mammalian species were observed in SC (Table S1). Species richness and abundance of individuals were clearly higher in forest patches than in shrub or open patches, and higher in shrub than open patches in both localities (Table S1).

DISCUSSION

The results of the seeding experiment indicate that the recruitment of five of the six studied species was facilitated by canopies, whether of trees or shrubs. Also, considering the total abundance of all the species combined as well as species richness, recruitment was higher under a woody canopy than in open sites. Similarly, the results of the natural generation experiment suggest woody patches facilitate regeneration, especially of forests. These results are consistent with those of other studies performed in central Chile (Fuentes et al. 1984, 1986, Armesto & Pickett 1985, Bustamante Holmgren et al. 2000, Becerra & Montenegro

2013, Cuevas et al. 2013, Velasco & Becerra 2020), as well as several works from other regions with semiarid climates (Callaway 1992, Pugnaire et al. 1996, Bonet 2004, Gómez-Aparicio et al. 2005, Maestre et al. 2009, Lloret & Granzow de la Cerda 2013, Caldeira et al. 2014).

On the other hand, our results suggest that woody regeneration coming from germination *in situ* was affected by herbivores only in some few species and patch types. However, these effects did not occur in terms of total abundance nor species richness. Whether the herbivory observed in this study was by seed or plant predators or both, the results suggest that herbivory does not have significant effects on total abundance or species richness that naturally regenerates in central Chile. Our results differ from those of a series of studies performed in regions where the European rabbit is present, such as central Chile (Fuentes et al. 1983, 1984, Holmgren 2002, Holmgren et al. 2006, Cuevas et al. 2013, Morales et al. 2015), and other regions of the world (Moreno & Villafuerte 1995, Bird et al. 2012), all of which suggest that woody regeneration is strongly affected by rabbits. These different findings may be related to two factors. First, these works have assessed the effects of rabbits with experimentally planted seedlings (not from germination *in situ*), with and without exclusion, and then monitoring for seedling predation by rabbits. Rabbits may prey on or simply cut woody seedlings of different species when the plants appear suddenly in their habitat (through planting) (personal observation), while they apparently browse less on woody seedlings that emerge from germination *in situ*. Second, the experimental designs used to study herbivory by rabbits on woody seedlings in this region (Fuentes et al. 1983, 1984, Holmgren et al. 2006, Cuevas et al. 2013, Morales et al. 2015) and other regions (Bird et al. 2012) only indicate the occurrence of the interaction and the level of predation on some species, which does not necessarily represent the impact on total abundance and species richness of woody species that naturally regenerate in a locality. In fact, the few studies evaluating the effect of rabbits on natural regeneration of woody species have not found an important impact of herbivory (e.g. Tiver & Andrew 1997, Travers et al. 2017).

On the other hand, in the few cases where we observed a significant effect of herbivory on the recruitment in the seeding experiment, part of these effects could be due to seed predation. Although few studies have evaluated seed predation in the study region, all of them indicate that effectively seed predation occurs on some woody species of central Chile (Bustamante & Vásquez 1995, Henríquez & Simonetti 2001, Celis et al. 2004, Cuevas et al. 2013, Morales et al. 2015). Nevertheless, the fact that natural regeneration in our study did not vary between the exclusion treatments suggests that even though predation of seeds of different species is occurring in central Chile, the excluded seed predators do not appear to affect natural regeneration importantly.

The observed effect of excluding herbivores on regeneration only varied among patch types in the seeding experiment and only in one of the studied localities (SC). In contrast, the exclusion of herbivores did not affect natural regeneration in any type of patch in any of the localities. These results suggest that the type of patch influences the level of herbivory, although this does not translate into an impact on natural regeneration. In the seeding experiment, predators reduced recruitment in forest patches and open sites, but not in shrub patches. These results are not completely consistent with what has been documented with respect to the effect of rabbits on different types of vegetation patches in central Chile.

Herbivory by rabbits is mainly observed in open or shrub areas (Fuentes et al. 1983). It is possible that the significant effects of herbivory on recruitment observed in the seeding experiment in open sites at the locality SC were the result of rabbits, which is consistent with the high frequency of observations of rabbits in open sites. However, there also was a high frequency of other herbivores, especially cows, in open sites in this locality, therefore, cows may also be responsible for the significant effect of exclusion observed in open sites. In contrast, the significant effect of herbivores on recruitment in forest patches could be result of seed predators as well as cows. Correa-Solís & Becerra (unpublished data) observed more seed predation in forest patches than the other patch types, which is consistent with the greater use of forest patches by potential seed predators observed in the locality SC. A large number of cows were also observed in forest patches in this locality.

The absence of effects of herbivory in shrub patches in SC is interesting because it is related to the lower frequency of visits to this type of patch by cows or rabbits (although this also occurred in the locality of Peñuelas). Considering the exclusion areas (without herbivores), there were no differences in recruitment between shrub patches and open sites in the seeding experiment, suggesting that the shrub patches do not significantly improve abiotic conditions that facilitate regeneration. This was also observed in the natural regeneration experiment. In the non-exclusion areas (with potential herbivore access to seeds and plants), recruitment in the seeding experiment was higher in shrub patches than in open sites, which could be the result of the lower presence of herbivores in shrub patches. This suggests that the shrub patches have a indirect positive effect on recruitment by reducing herbivory by cows and rabbits. Other works have also observed that the regeneration of woody species can be facilitated indirectly through reducing the level of herbivory (Bertness & Callaway 1994, Smit et al. 2006).

Our results show that natural regeneration of woody species in central Chile is more affected by the facilitation provided by tree or shrub canopies than by seedling and seed predation, although some species in the region are clearly subject to herbivory. While shrub patches facilitate regeneration indirectly by reducing the level of herbivory, it is likely that the main facilitation mechanism of woody vegetation, especially in forest patches, is related to reduced abiotic stress, especially water stress (Velasco & Becerra 2020). Several works have suggested that the main facilitation mechanism from shrubs and trees on regeneration of woody species in semiarid climates is improving soil moisture conditions by providing shade (Rey-Benayas et al. 2005, Sánchez-Gómez et al. 2006, Maestre et al. 2009, Caldeira et al. 2014, Saura-Mas et al. 2015), although improving other soil conditions may also have a role (Gómez-Aparicio *et al.* 2005).

Natural regeneration in open sites in the studied localities was extremely low, with few species present (Table S2), which suggests that recolonization and successional advance will be very slow. The species observed in open sites were mainly recognized pioneering species like *A. caven* and *K. oblonga* (Armesto & Pickett 1985, Fuentes et al. 1986), as well as species like *Q. saponaria* and *M. boaria*, which, although normally regenerate under canopies, in localities with more moisture (like LP and SV) may recruit in open sites (Holmgren et al. 2000). The number of species in shrub patches was slightly higher. However, the species that regenerated in these patches are ones normally considered as pioneers like *Baccharis spp.* and *Retanilla trinervia*, with no regeneration of late successional species like *P. boldus, C. alba,* and *L. caustica* (Table S2). This also suggests that the advance to late or climax stages will be slow or nil. The largest number of regenerating species were found in the forest patches, which was the only patch type where late successional species were found regenerating. These patches also presented regeneration of several pioneer species (*S. polygamus, B. paniculata. K. oblonga*) (Table S2). Our results suggest that the slow or almost nil successional advance from open or shrub patches to forest patches with climax species is not the result of herbivory, or that the effect of herbivory is very weak. It is likely that the decreased precipitation related to climate change that has experienced in central Chile during the last decades (Garreaud et al., 2020) is one of the main causes of successional stagnation. Other works have already shown that tree canopies are necessary for regenerating woody species in Mediterranean-type ecosystems (e.g. Lloret et al. 2005, Bhatta & Vetaas 2016), which also suggests that the recovery of woody vegetation in open sites is extremely slow and poor in terms of species diversity.

Our results can have important implications for reforestation and plant restoration. Given the strong negative effects of rabbits on planted woody seedlings that have been documented in central Chile and other regions (Fuentes et al. 1983, 1984, Holmgren et al. 2006, Cuevas et al. 2013, Morales et al. 2015, Bird et al. 2012), the fact that herbivory has little or no effect on regeneration from germination *in situ*, suggests that sowing may be a more economical way to reforest, especially on large spatial scales, since it would not be necessary fences or exclosures. Our results also suggest that to restore late successional stages, recovery of pioneer stages of shrub patches is not enough, because these patches do not significantly facilitate the natural regeneration of late successional woody species.

Consequently, the restoration of late successional stages does not occur passively (through natural regeneration) in pioneer stages of vegetation. It is probable that more active treatments are required to restore forest patches.

In conclusion, our results suggest that the natural regeneration of woody species in central Chile is facilitated by the canopies of woody species, especially in forest patches. Vertebrate herbivores affect the recruitment of some woody species, which occurs mainly in forest patches and open sites. However, herbivory does not appear to have a significant impact on the levels of abundance or species richness of natural regeneration.

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Author contributions

PB conceived and designed the experiments. MJC performed the experiments. PB and MJC analyzed the data and wrote the manuscript.

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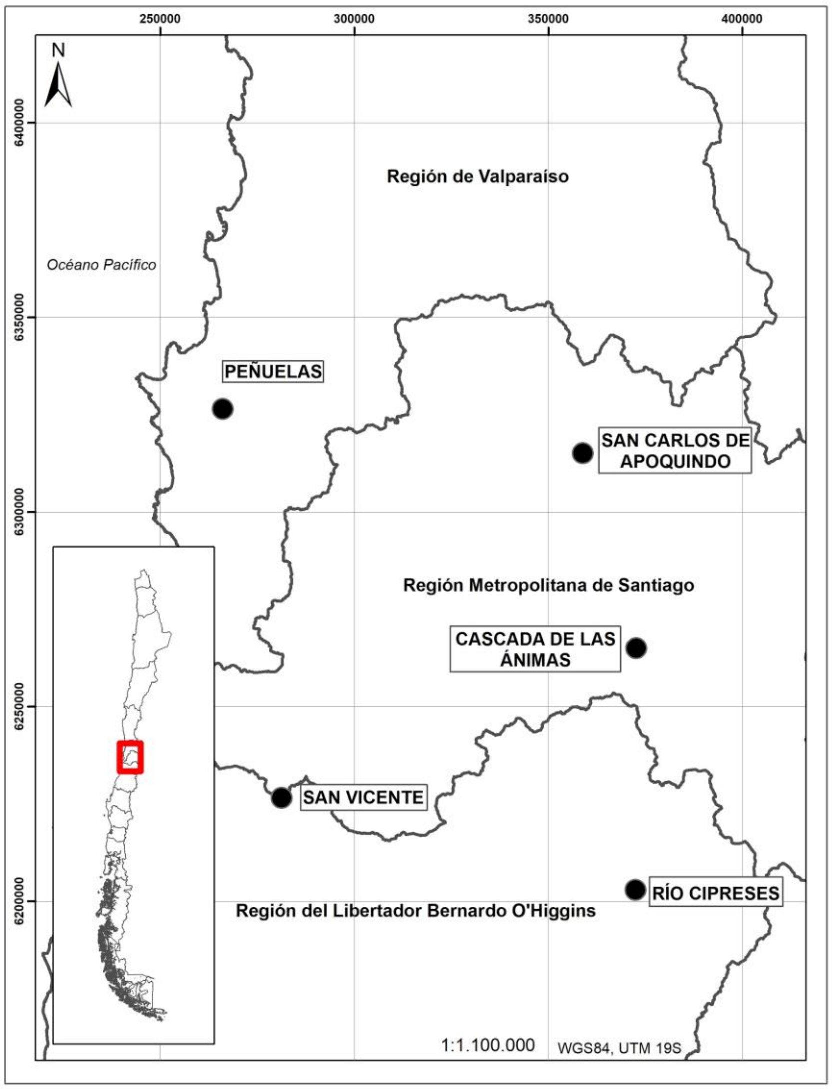

Figure 1. Geographic locations of the studied sites

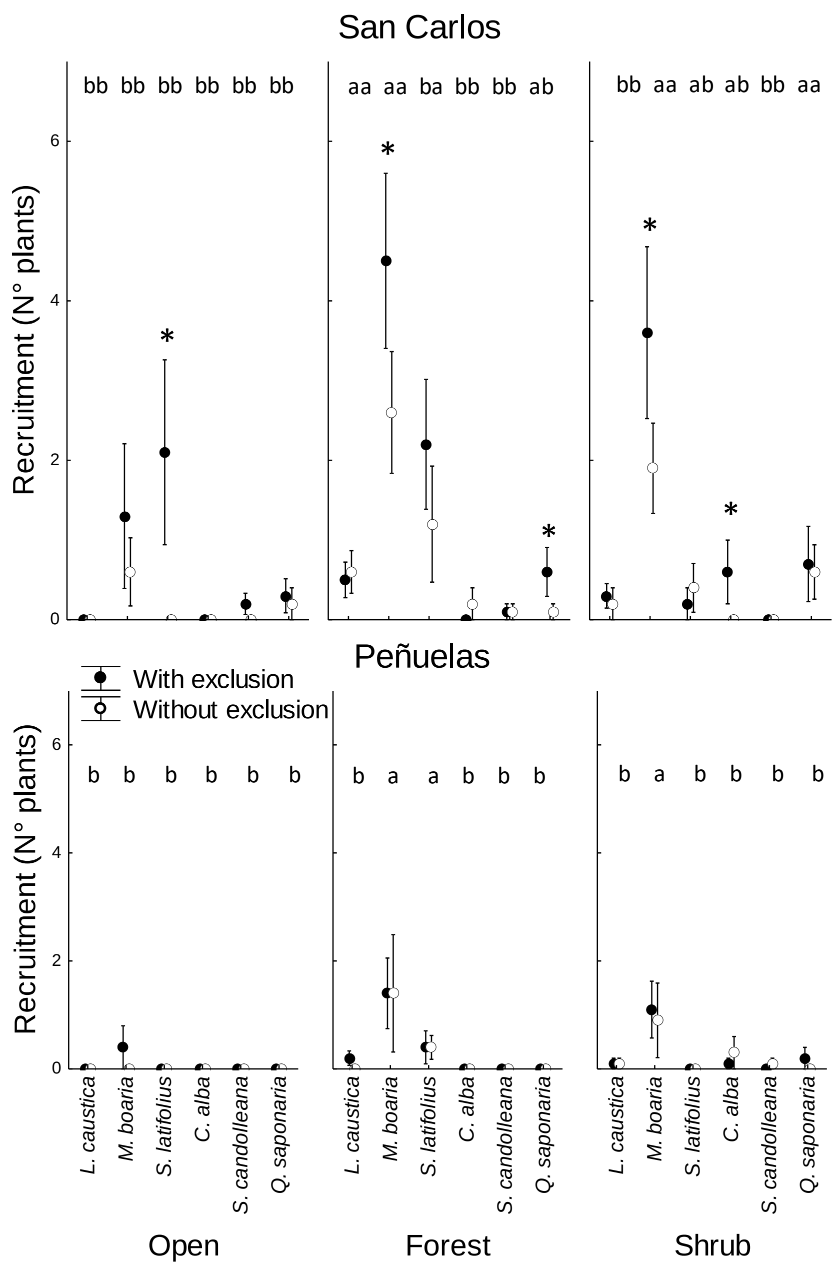

Figure 2. Recruitment from the experimental sowing observed in each locality, patch type, exclusion treatment and species. Asterisks indicate significant differences (GLM, P<0.05) between exclusion treatments. Different letters indicate significant differences (GLM, P<0.05) between patch types for a single locality, exclusion treatment and species. In the case of the locality of Lago Peñuelas, different letters indicate significant differences between patch types combining both exclusion treatments.

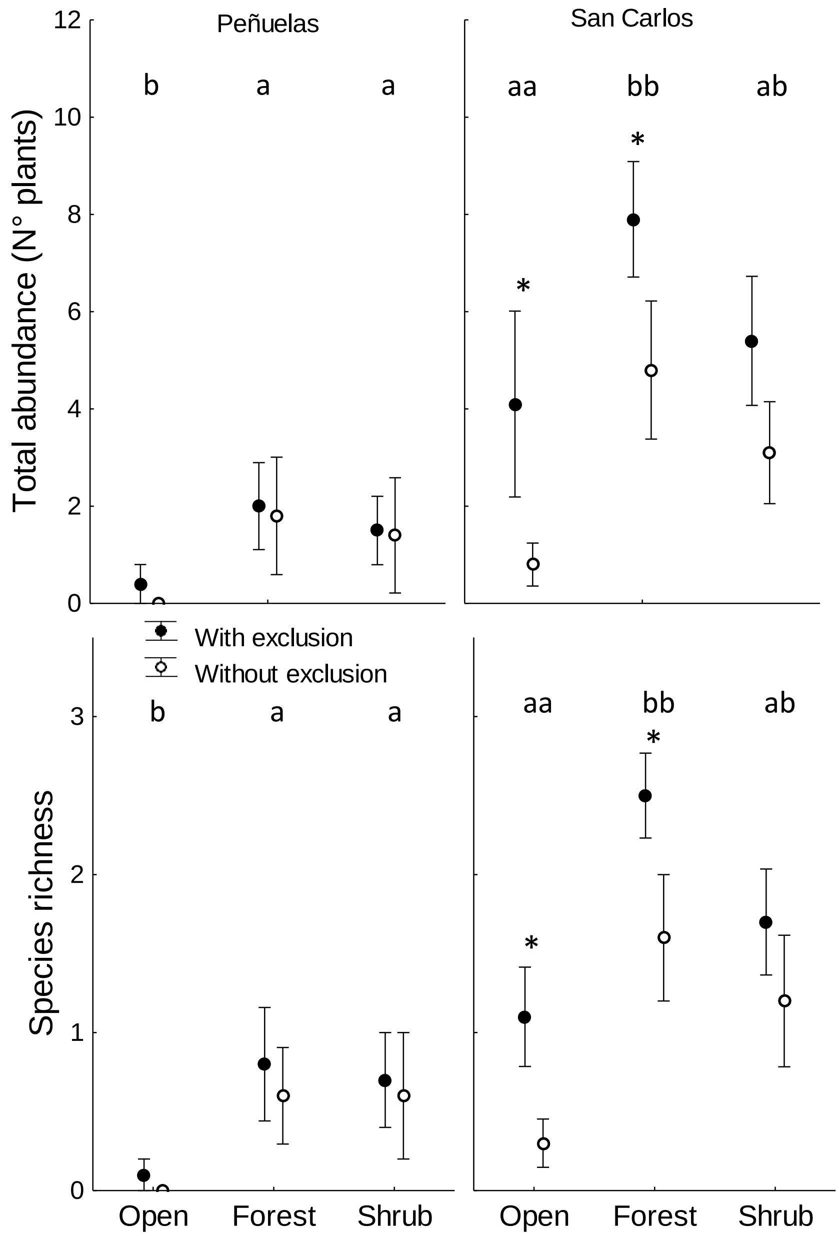

Figure 3. Recruitment from the experimental sowing (total abundance combining all species and species richness) observed in each locality, patch type and exclusion treatment. Asterisks indicate significant differences (GLM, P<0.05) between exclusion treatments. Different letters indicate significant differences (GLM, P<0.05) between patch types for a single locality and exclusion treatment. In the case of the locality of Lago Peñuelas, different letters indicate significant differences between patch types combining both exclusion treatments.

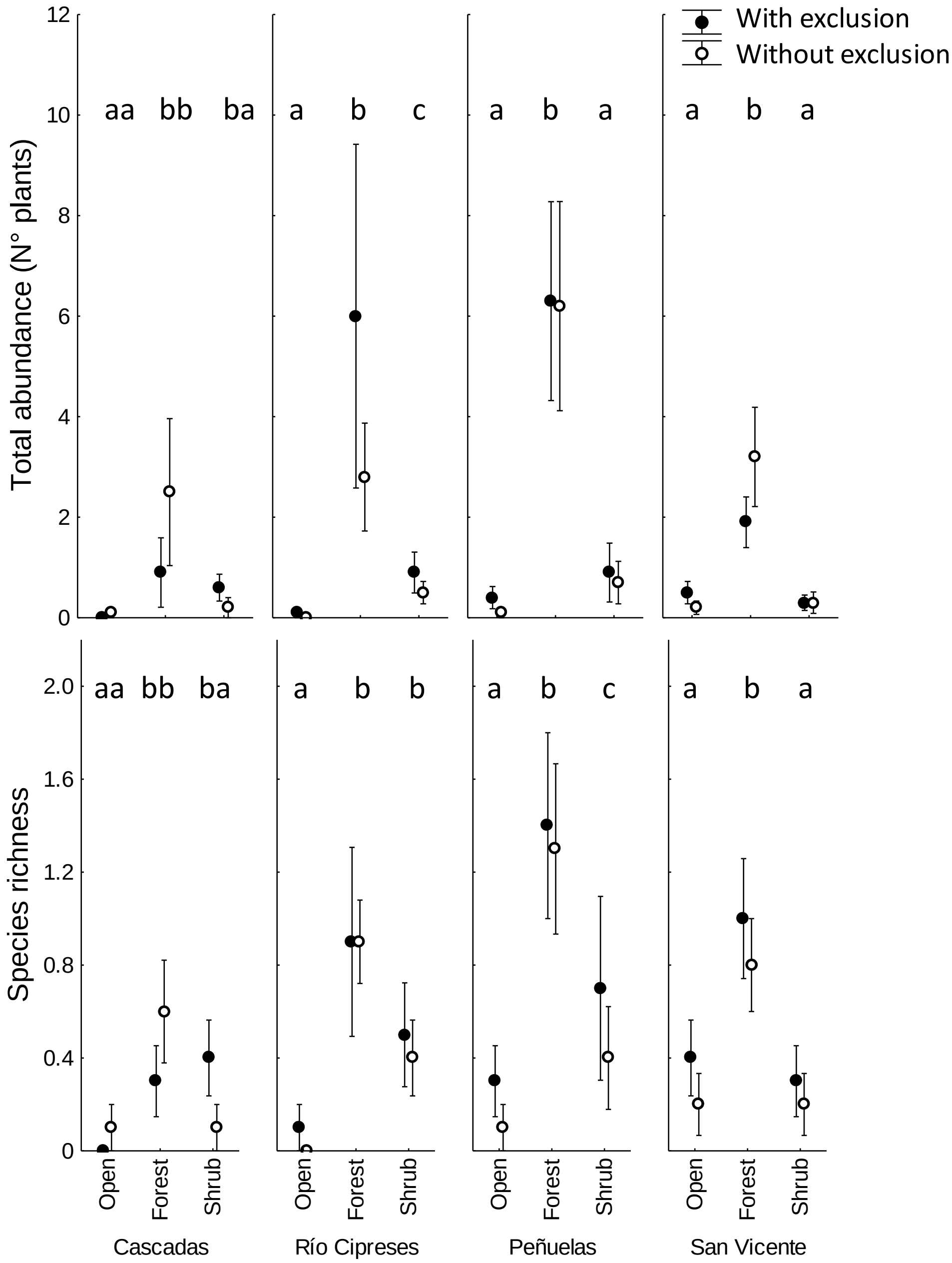

Figure 4. Natural regeneration observed (total abundance combining all species and species richness) in each locality, patch type and exclusion treatment. Different letters indicate significant differences (GLM, P<0.05) between patch types for a single locality, combining both exclusion treatments, except in the locality of Cascadas, where different letters indicate significant differences between patch types for a single exclusion treatment separately.

Table 1. Statistical results (GLM) for the effects of patch type, exclusion treatment and species on the number of recruited plants from the seeding experiment in both localities (significant values are in bold).

	Lago Peñuelas (LP)		San Carlos (SC)	
	Chi-square	P	Chi-square	P
Patch	16.310	**<0.001**	38.232	**<0.001**
Exclusion	0.707	0.400	28.419	**<0.001**
Species	31.295	**<0.001**	288.502	**<0.001**
Patch*Exclusion	1.140	0.565	7.797	**0.020**
Patch*Species	14.092	**0.003**	47.894	**<0.001**
Exclusion*Species	4.495	0.343	2.318	0.804
Patch*Exclusion*Species	1.360	0.715	25.768	**<0.001**

Table 2. Statistical results (GLM) for the effects of patch-type and exclusion on total abundance (combining all species) and recruited species richness from the seeding experiment in the two studied localities (Significant values are in bold).

	Lago Peñuelas (LP)				San Carlos (SC)			
	Total abundance		Species richness		Total abundance		Species richness	
	Chi-square	P	Chi-square	P	Chi-square	P	Chi-square	P
Patch	7.874	**0.020**	15.501	**<0.001**	35.869	**<0.001**	20.549	**0.008**
Exclusion	1.096	0.295	0.573	0.449	29.562	**<0.001**	10.552	**0.032**
Patch*Exclusion	0.950	0.622	1.177	0.555	8.697	**0.013**	14.759	**0.005**

Table 3. Statistical results (GLM) for the effects of the patch type and exclusion on total abundance (combining all species) and plant species richness from natural regeneration in the studied localities.

	Cascadas (CA)		Río Cipreses (RC)		Lago Peñuelas (LP)		San Vicente (SV)	
	Chi-square	P	Chi-square	P	Chi-square	P	Chi-square	P
Total abundance								
Patch	8.767	**0.012**	19.944	<**0.001**	177.477	<**0.001**	58.075	<**0.001**
Exclusion	0.127	0.722	0.007	0.932	0.247	0.619	1.569	0.210
Patch*Exclusion	5.107	0.078	2.273	0.321	1.939	0.379	3.110	0.211
Species richness								
Patch	8.052	**0.018**	18.522	<**0.001**	20.138	<**0.001**	10.065	**0.007**
Exclusion	0.016	0.900	0.143	0.705	0.860	0.354	0.866	0.352
Patch*Exclusion	5.003	0.080	1.355	0.244	1.052	0.591	0.237	0.888

Table S1. Community of potential herbivores (of seeds and/or seedlings) observed in two localities included in the study. The documented or observed diet of the species (F=Fruit, Fu=Fungus, I= Insects, N=Nectar, S=Seeds, V=vegetative structures, O=omnivore species), abundance (number of individuals) and species richness registered in the patch types per locality are indicated (*: introduced species).

Scientific name	Diet	Lago Peñuelas (LP)			San Carlos (LP)		
		Open	Shrub	Forest	Open	Shrub	Forest
BIRDS							
Anairetes parulus	I, S	0	2	10	0	2	3
Callipepla californica *	S, I	0	32	36	0	32	45
Columbina picui	S	0	0	0	0	0	2
Curaeus curaeus	S, I, F	10	11	38	3	25	37
Diuca diuca	S, I	1	8	13	1	1	8
Leistes loyca	I, F, S	25	17	13	18	11	8
Mimus thenca	I, F, S, N	5	5	12	3	5	11
Molothrus bonariensis	S, I	0	0	0	17	16	23
Nothoprocta perdicaria	S, I	0	1	2	0	0	2
Phytotoma rara	V	4	7	12	1	1	6
Phrygilus fruticeti	S, I, F	0	8	21	0	8	11
Phrygilus gayi	S, I	0	1	2	0	0	1
Sicalis luteola	S	0	16	18	0	17	23
Spinus barbata	S	0	3	4	0	3	5
Zenaida auriculata	S	8	10	21	6	8	20
Zonotrichia capensis	I, F, S	0	9	16	2	4	11
MAMMALS							
Abrothrix olivaceus	V, S, Fu	0	0	5	0	0	0
Bos taurus *	V	41	36	34	32	10	28
Equus asinus *	V	2	0	0	0	0	0
Equus caballus *	V	9	5	0	0	2	0
Lama guanicoe	V	4	9	0	0	0	0
Lycalopex culpaeus	O	1	5	17	1	0	0
Oryctolagus cuniculus *	V	4	4	1	13	0	4
Thylamys elegans	O	0	0	0	0	0	2
Rattus norvegicus *	O	0	0	2	0	0	3
Species richness		12	19	19	11	15	20
Total observed abundance		114	189	277	97	145	253

Table S2. Composition and abundance of species observed regenerating naturally within (W) and outside (O) the exclosures in each patch type and locality. Values indicate the total number of plants observed among all the replicates.

Scientific name	Cascada de las Ánimas						Río Cipreses						Lago Peñuelas						San Vicente					
	Open		Shrub		Forest		Open		Shrub		Forest		Open		Shrub		Forest		Open		Shrub		Forest	
	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O	W	O
Acacia caven							1	0											2	0	1	0		
Aristotelia chilensis											1	0												
Azara petiolaris											1	2												
Baccharis concava									2	1														
Baccharis paniculata									4	0													1	0
Cryptocarya alba											4	8					1	2						
Gochnatia foliolosa									1	2														
Kageneckia oblonga	0	1	2	0	8	3			2	1														
Lithraea caustica					0	1					51	12											1	0
Maytenus boaria			4	2	1	7			0	1	2	6	4	1	4	6	50	55	2	1	0	3	13	34
Peumus boldus					0	1									1	0	4	1					0	4
Quillaja saponaria															3	1	7	3	1	1			1	0
Retanilla trinervia																					1	0		
Schinus latifolius															1	0	1	1					1	0
Schinus polygamus											1	0												
Species richness	0	1	2	1	2	5	1	0	4	4	6	4	1	1	4	2	5	5	3	2	2	1	5	2
Total abundance	0	1	6	2	9	14	1	0	9	5	60	28	4	1	9	7	63	62	5	2	2	3	17	38

2. General Discussion

Seed herbivory experiments (Chapter 1) suggest that the exclusion of seed predators significantly reduced seed removal in all patches and localities in most species, being only insignificant in open areas in certain species (*S. latifolius, P. boldus* and *S. candolleana* in San Carlos, and *A. caven* in Peñuelas). In the seed removal experiment at the community level, herbivore exclusion significant increased the total number of seeds as well as species richness available to germinate and recruit. This was observed in all vegetation patches (Chapter 1). In addition, when comparing predation by patch type, we found that seed removal in terms of total seed abundance and species richness was significantly greater under the forest canopy, than in shrub and open areas. However, in the seed bank experiment, the effect of predators was only observed in forest and shrub patches, and not in all localities studied (Chapter 1).

The results of the sowing experiment (Chapter 2) show that, when compared to open sites, seedling recruitment of several species was facilitated by a tree canopy and a shrub canopy. At the community level, with regard to total abundance and species richness, regeneration was facilitated by forest and shrub patches. A similar patch type effect was observed in the natural regeneration experiment (Chapter 2), total abundance was greater under a forest canopy than under shrub and open areas. On the other hand, although herbivore exclusion increased recruitment of some species as well as total abundance and species richness, herbivory had no significant effect on natural regeneration.

Adult vegetation and the presence of herbivores can significantly affect the natural regeneration of plants, as well as that of reforestation processes (De Steven 1991, Crawley 1992, Hanley 1998). In degraded semi-arid ecosystems, where vegetation appears mainly as patches that vary in coverage and height, regeneration from *in-situ* germination (either natural or from sowing) is frequently facilitated by patches with greater woody coverage. These patches provide better physical microhabitat conditions for seedling germination and survival (Armesto & Pickett 1985, Callaway 1992, Pugnaire et al. 1996, Bonet 2004, Gómez-Aparicio et al. 2005, Maestre et al. 2009, Lloret & Granzow de la Cerda 2013, Caldeira et al. 2014). However, the type of patch can also modulate the effect of herbivores, by affecting their frequency and diversity (Crawley 1992, Guiden & Orrock 2017, Wang et al. 2019).

Seed predation experiments (Chapter 1) revealed that this process is in fact, occurring in several species in central Chile, significantly reducing abundance and to a lesser extent, richness of seeds available for germination and recruitment. Our results also suggest that the predation is namely generated by birds and rodents. Previously, though in only a few woody species, a number of studies carried out in Chile's central region documented similar results (*C. alba, B. miersii*; Bustamante & Simonetti 2000, Henríquez & Simonetti 2001, Celis et al. 2004, Morales et al. 2015). Other works also reported similar results in temperate forests of south-central Chile

(Díaz et al. 1999, Donoso et al. 2003). Furthermore, the results of this study suggest that, although the exclusion of seed predators resulted in reduced seed predation in all patch types and species, the level of seed predation was higher in forest and scrub patches. This was also expressed in the seed bank as a significant effect of herbivore exclusion was observed in forest or shrub patches in the seed bank of 4 of the 5 localities.

In the seedling regeneration experiments (Chapter 2), it was not possible to differentiate if the herbivory occurred on the seeds and/or seedlings. Although the results suggest that vertebrate herbivores could reduce regeneration by affecting seed or seedling stages, this process is variable between locations (this was observed in one of the two locations where it was experimentally evaluated). Furthermore, herbivory would not have a significant impact on natural regeneration in terms of total abundance or species richness. These results are not consistent with a series of studies developed in central Chile on seedlings, regarding the role of herbivory generated by rabbits. These studies noted that rabbits prey on plants of different woody species in this region (Fuentes et al. 1983, 1984, Holmgren 2002, Holmgren et al. 2006, Cuevas et al. 2013, Morales et al. 2015). However, all previous studies mentioned, have evaluated the effect of rabbits on experimental plant planting, which differs from the present study carried out on natural regeneration. Rabbits may prey upon, or cut plants of the various native species, when the plants have appeared in their habitat unexpectedly (through planting), and it is also possible that rabbits may forage less, when plants originate from *in-situ* germination.

On the other hand, the experimental designs used in herbivory studies generated by rabbits in this area (Fuentes et al. 1983, 1984, Holmgren et al. 2006, Cuevas et al. 2013, Morales et al. 2015) only allow conclusions about the occurrence of the interaction, and level of predation on some species. Yet, this does not necessarily represent the impact on total abundance, or richness of woody species that regenerates naturally. Nonetheless, in this same study (Chapter 2), the exclusion effect of herbivores was significant in forest and open site patches, in some planted species (only in one of the two localities). It is likely that the positive effect of herbivore exclusion on *in situ* regeneration from experimental sowing in forest patches, was caused by increased seed predation in forest patches (Chapter 1). On the other hand, the significant effect of herbivore exclusion on recruitment in open sites, was probably caused by rabbits and/or livestock, and their greater use of open spaces (Fuentes et al. 1983).

Despite the effects herbivores may have on plant regeneration, our results indicate that in central Chile, herbivores, either preying on seeds or seedlings, show a preference for certain vegetation patches. Microhabitat characteristics can influence animal behavior, as well as the presence, abundance and even animal community arrangement (Morris & Davidson 2000, Mabry & Stamps 2008). Consequently, interactions between plants and animals may depend on the qualities of the microhabitats (Chase 1998, Rey et al. 2002, Orrock et al. 2010, Silliman et al. 2013, Mezquida & Benkman 2014). Vegetation cover and patch type can modulate the presence and abundance of herbivores (Díaz et al. 1999, Kelt et al. 2004). Our results are consistent with these observations. We observed greater species richness and abundance of herbivorous in forest than shrub patches, and also greater in shrub than in open sites (Table 1). This was repeated in the two localities studied, suggesting that this would be a common pattern within the central Chile region. The use of microhabitats by local fauna may be explained by a number of factors. It could be related to having better protection against their predators and refuge provided by patches with greater woody cover (Brown & Ojeda 1987, Kelt et al. 2004). Also, this preference could be related to higher concentrations of seeds (a significant proportion of the food of herbivores studied Table 4, Chapter 1, Table S1, Chapter 2) under woody patches, probably resulting from the highest seed dispersal under woody patches. In our study (Chap. 1) the seed bank within the exclusions, was always greater in shrub or forest patches, rather than in open sites. This was also previously described by Jiménez & Armesto (1992). In our study areas, it was noted that herbivores are species that predominantly inhabit forest or shrub environments, including exotic species (*Rattus norvegicus, Callipepla californica*). Similarly, other studies have described greater seed predation in larger forest fragments than in smaller patch or fragment edges (Burkey 1993, Díaz et al. 1999).

When the results of the two studies in this thesis are consolidated (Chapters 1 and 2), it can be concluded that in central Chile, an important number of woody plant species are subject to seed predation. It is likely that most of this predation is greater in forest patches, than in other patch types. However, seed predation that occur, does not have a significant and widespread impact on plant recruitment. The results show that in certain localities, herbivory may have a significant effect on the regeneration of some woody species either from seeds or seedlings. This would however, not be geographically widespread in central Chile and would probably not have a significant impact on natural regeneration. In addition, as it has been noted in previous studies, herbivory on seedlings and plants may occur in some species of central Chile. However, this would not have a significant impact on natural regeneration. In contrast, our research shows that natural regeneration of woody species in central Chile is most affected by the facilitation processes of shrubs or trees. That is, even though most herbivory occurs in patches with the greatest regeneration (Forest> Shrub> Open), the facilitation mechanisms generated by vegetation, outweigh the effects of herbivores. In conditions of vertebrate herbivore exclusion, *in situ* regeneration whether natural or from sowing processes was significantly and positively influenced by woody cover. These results are consistent with other studies carried out in Chile´s central region (Fuentes et al. 1984, 1986, Armesto & Pickett 1985, Holmgren et al. 2000, Becerra & Montenegro 2013, Cuevas et al. 2013, Velasco & Becerra 2020), as well as with studies carried out in semi-arid regions (Callaway 1992, Pugnaire et al. 1996, Bonet 2004, Gómez-Aparicio et al. 2005, Maestre et al. 2009, Lloret & Granzow de la Cerda 2013, Caldeira et al. 2014). In all likelihood, the main facilitation mechanism generated by woody patches is related to a reduction of abiotic stress, particularly water stress (Velasco & Becerra 2020). Previous work further suggests that improved humidity levels due to shading, is the principal mechanism for facilitating the effect of shrubs or trees on woody plants’ regeneration in semi-arid climates (Rey-Benayas et al. 2005, Sánchez-Gómez et al. 2006, Maestre et al. 2009, Caldeira et al. 2014, Saura-Mas et al. 2015). However, this may also be the result of effects that are generated by the woody canopy on the ground (Gómez-Aparicio et al. 2005).

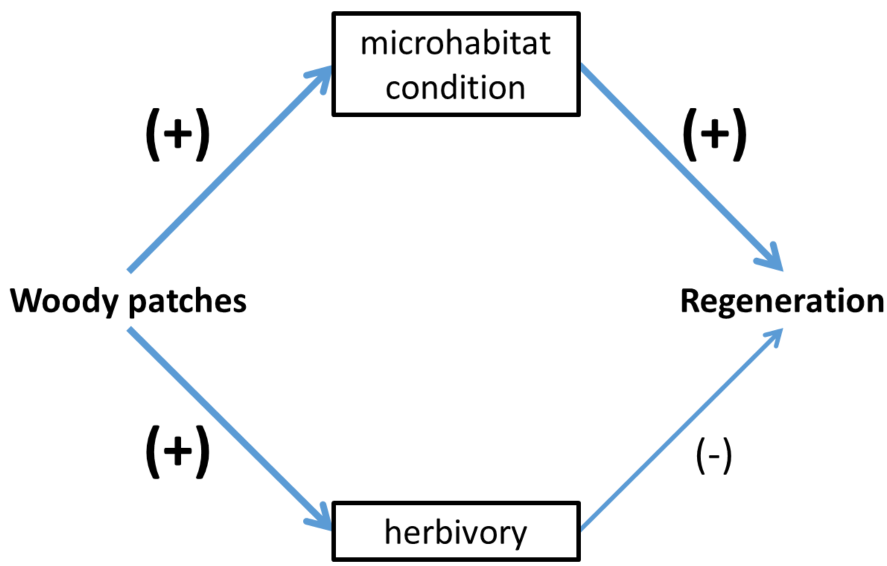

Figure 1. Diagram modified from the one presented in Figure 1 of the General Introduction, emphasizing the relative magnitude (width of the arrows) and direction (-, +) of the effects of the patches of vegetation and herbivores observed in the present investigation.

Our results may have important implications for reforestation and vegetation restoration strategies. The fact that herbivory has little effect on *in situ* regeneration, either natural or from sowing (unlike the effects observed by other herbivory work on plants through planting), suggests that sowing could be a more effective and financially sound form of reforestation that may be applicable on large spatial scales.

However, our results also suggest that in order to restore advanced successional stages, recovering the pioneering stages of shrub patches would not be sufficient. This is based on the fact that these do not facilitate large scale natural regeneration of advanced successional woody species. Furthermore, there is no apparent recovery of pioneering shrub patches, either through natural regeneration or sowing. Hence, restoration of late-succession species would not occur passively (natural regeneration), or from sowing. This would in all likelihood require active measures to restore forest patches, namely the improvement of moisture in the soil. For instance, to allow higher humidity levels for seeds, sowing should be buried, or under leaf litter. Additionally, irrigation may be used, depending on seasonal rainfall levels and available financing. Late-succession (forest) species can regenerate and self-sustain their populations, only once a forest cover has been established.

3. Conclusions

It is possible to conclude that in Chile central seed predation occurs in a significant number of woody plant species, and this predation is greater in forest patches than in other types of patch. However, this does not have a significant and widespread impact on plant recruitment. Our results also suggest that, although herbivory on seedlings may occurs on several woody species, such as has been documented in literature, this herbivory would not have a significant negative impact on natural regeneration. Instead, natural regeneration in central Chile is more strongly controlled by facilitation processes generated by the presence of shrubs or trees, even though seed predation may be stronger in forest or shrub patches.

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