Recruitment limitations in *Primula vulgaris* in a fragmented landscape

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Abstract

Plant recruitment is limited by dispersal, if seeds cannot arrive at potential recruitment sites, and by establishment, due to a low availability of safe sites for recruitment. Seed-sowing experiments, scarcely applied along gradients of landscape alteration, are very useful to assess these limitations. Habitat loss and fragmentation may foster recruitment limitations by affecting all the processes from seed dispersal to seedling establishment. In this study, we perform a seed-sowing experiment to disentangle the importance of dispersal and establishment limitations in different stages of recruitment of the perennial herb *Primula vulgaris* in fragmented forests of the Cantabrian Range (Northwestern Spain). We evaluated the influence of ecological gradients resulting from habitat loss and fragmentation (modifications of habitat amount at the landscape and microhabitat scales, changes in the species' population size, changes in seed predation and seedling herbivory) on seedling emergence, survival and early growth. We found strong evidence of dispersal limitation, as seedling emergence was very low in experimental replicates where no seeds were added. This limitation was independent of landscape alterations, as we found no relation with any of the ecological gradients studied. Establishment limitations at the germination phase were also unrelated to ecological gradients, probably because these limitations are more related to fine-scale environmental gradients. However, further monitoring revealed that seedling survival after summer and winter periods and seedling growth were conditioned by landscape alteration, as we found effects of habitat amount at the landscape and microhabitat scales, of presence of populations of *P. vulgaris* and of seedling herbivory. These effects were complex and sometimes opposite to what can be expected for adult plants, revealing the presence of different requirements between life stages.

Zusammenfassung

Die Ansiedlung von Pflanzen wird durch die Ausbreitung begrenzt, wenn Samen nicht zu einem möglichen Ansiedlungsstandort gelangen können, und durch die Ansiedlung selbst aufgrund der geringen Verfügbarkeit von sicheren Standorten für die Ansiedlung. Aussaatexperimente sind sehr nützlich, um diese Begrenzungen festzustellen, werden jedoch selten entlang von Gradienten landschaftlicher Veränderungen angewendet. Habitatverluste und Fragmentierung können die Ansiedlungslimitierung verstärken, indem sie alle Prozesse von der Samenverbreitung bis zur Ansiedlung der Keimlinge beeinflussen. In dieser Untersuchung führten wir ein Aussaatexperiment durch, um den Einfluss der Verbreitungs- und der Ansiedlungslimitierungen in verschiedenen Stadien der Ansiedlung des perennierenden Krauts *Primula vulgaris* in fragmentierten Wäldern des Kantabrischen Gebirges (Nordwest-Spanien) zu untersuchen. Wir evaluierten den Einfluss der ökologischen Gradienten, die aus dem Habitatverlust und der Fragmentierung resultieren (Veränderungen des Habitatanteils auf der Landschafts-und der Mikrohabitat-Skala, Veränderungen in der Populationsgröße der Art, Veränderungen bei Samenprädation und Keimlingsfraß), auf das Auflaufen der Keimlinge, die Überlebensrate und das frühe Wachstum. Wir fanden starke Hinweise auf eine

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Ausbreitungslimitierung, da es nur ein geringes Auflaufen von Keimlingen bei experimentellen Standorten gab, bei denen keine Samen hinzugefügt wurden. Diese Begrenzung war unabhängig von den landschaftlichen Veränderungen, da wir keine Beziehung zu irgendeinem der untersuchten ökologischen Gradienten fanden. Die Ansiedlungslimitierung in der Keimphase war ebenfalls von den ökologischen Gradienten unabhängig, vermutlich weil diese Begrenzungen eher mit Umweltgradienten auf der kleinräumigen Skala in Beziehung stehen. Durch das weitergeführte Monitoring stellte sich jedoch heraus, dass die Überlebensrate der Keimlinge nach den Sommer- und Winterperioden und das Keimlingswachstum durch die Veränderungen der Landschaft beeinflusst wurden, da wir einen Einfluss des Habitatanteils auf der Landschafts- und Mikrohabitatskala, der Anwesenheit von bestehenden P. vulgaris-Populationen und der Samenprädation fanden. Diese Auswirkungen waren komplex und standen manchmal im Gegensatz zu dem, was für erwachsene Pflanzen zu erwarten ist, und sie zeigten so, dass es unterschiedliche Anforderungen in den unterschiedlichen Lebensstadien gibt.

Introduction

Seedling recruitment is a key step of plant life cycles, and it determines plant distribution, population growth and colonization of new areas (Ehrlén & Eriksson 2000; Wang & Smith 2002). Recruitment can be hampered by two sequential limitations (Baeten et al. 2009): *dispersal limitation* (or *seed limitation*), due to the failure of seeds to arrive at saturating densities at potential recruitment sites (Eriksson & Ehrlén 1992; Nathan & Muller-Landau 2000), and *establishment limitation* (or *microsite limitation*), due to a low availability of safe sites for establishment once seeds have arrived (Clark, Macklin, & Wood 1998; Nathan & Muller-Landau 2000). Disentangling the relative importance of both limitation types is essential to understand plant population response to environmental variability (e.g. Kolb & Barsch 2010; Rother, Jordano, Rodrigues, & Pizo 2013). To that end, observing seedling emergence from experimentally added seeds in sites where they are naturally absent (by performing seed-sowing experiments; Clark, Poulsen, Levey & Osenberg 2007) indicates that recruitment is seed-limited, whereas seedling absence or losses after germination in those sites with seeds added would indicate establishment limitation.

Anthropogenic disturbances of the landscape, such as habitat loss and fragmentation, may exacerbate recruitment limitations, leading plant populations to severe decline or even extinction (Bruna, Fiske, & Trager, 2009). Landscape changes may affect differentially the processes of seed dispersal, germination and seedling establishment. For example, habitat fragmentation may reinforce dispersal limitation, by decreasing seed production after reducing plant population sizes or decreasing pollination success (Aguilar, Ashworth, Galetto, & Aizen 2006; González-Varo, Arroyo, & Aparicio 2009; Valdés & García 2011), or by hampering seed dispersal among habitat remnants (e.g. Cordeiro & Howe 2003). Moreover, establishment limitations may be fostered by the detrimental environmental conditions imposed by habitat loss and fragmentation through, for example, increased seed and seedling predation (Benitez-Malvido 1995; Farwig

et al., 2009), and abiotically restricted seed germination (Bruna 1999) and seedling survival (Uriarte, Bruna, Rubim, Anciães & Jonckheere 2010). Although there have been some experimental seed sowing studies assessing dispersal and establishment limitations (e.g. Baeten et al. 2009 and meta-analyses by Turnbull, Crawley, & Rees 2000 and Clark et al. 2007), they scarcely evaluate the relative importance of both limitation types in human-altered landscapes, and these studies are predominantly observational (Cordeiro & Howe 2003; Kunstler et al. 2007; McEuen & Curran 2004; Uriarte et al. 2010).

In this study, we perform a landscape-scale seed-sowing experiment in order to assess the importance of dispersal and establishment limitations in the recruitment of the perennial herb *Primula vulgaris* in a fragmented landscape of the Cantabrian range (Northwestern Spain). *P. vulgaris* behaves mainly as a forest species in this region and forest habitat loss has been stated to negatively affect its reproduction (Valdés & García 2011). Perennial forest herbs are a suitable group for the evaluation of the effects of habitat loss and fragmentation on recruitment, due to their high susceptibility to forest disturbance and their strong dispersal limitation (Honnay, Jacquemyn, Bossuyt, & Hermy 2005). We chose *P. vulgaris* as our study species because it is widespread in our study area and this allows to study its recruitment along wide landscape gradients. Moreover, spatial distribution of populations at a wide scale suggests the existence of dispersal limitations (Valdés & García 2009). We expect *P. vulgaris* recruitment to be sensitive to habitat loss and fragmentation due to lowered seed production, restricted dispersal and/or strongly modified habitat conditions for seedling establishment and growth (Valverde & Silvertown 1995; Jacquemyn, Endels, Brys, Hermy & Woodell 2009; Valdés & García 2011). Specifically, we seek to answer the following questions: (1) is seedling emergence of *P. vulgaris* limited by dispersal, establishment, or both? (2) how are seedling emergence, survival and early growth affected by the ecological gradients resulting from habitat loss and fragmentation (modifications of habitat amount, changes in the species' population size, changes in seed predation and seedling herbivory)?

Material and methods

Study area

Our study was carried out in Sierra de Peña Mayor, Asturias, Spain (43°17′ N–5°30′ W, 900 m a.s.l.), within the Cantabrian Mountains. Its climate is Atlantic, with a mean annual temperature of 13 °C and annual rainfall of ca. 1300 mm. Resulting from historical deforestation for cattle grazing, the landscape of this locality has evolved from extensive temperate forests to highly variegated woodland pastures.

The study area (Fig. 1) comprises a mosaic composed of a few large forest fragments with hardwood species (beech: *Fagus sylvatica* L. and ash: *Fraxinus excelsior* L.), a fringe of fleshy-fruited trees (holly: *Ilex aquifolium* L., hawthorn: *Crataegus monogyna* Jacq., yew: *Taxus baccata* L., rowan: *Sorbus aucuparia* and whitebeam: *Sorbus aria*) and hazel (*Corylus avellana* L.), numerous small forest fragments dominated by fleshy-fruited trees, and an extensive (>60% cover) matrix of stony pastures and heathlands (*Erica* spp., *Ulex europaeus* L.).

Study species

Our target species is *P. vulgaris* L. (Primulaceae), a perennial, early-flowering herb, typically living in moist open habitats in Europe (Endels, Jacquemyn, Brys, Hermy, & De Blust 2002), but restricted to temperate forests in some parts of its range. In the Cantabrian Mountains, it behaves mainly as a forest species, although it can be also found in some other semi-shaded locations, like roadsides and trails. It grows through the production of leaves in a basal rosette, with occasional vegetative spread through lateral rosettes. In our study area, flowering occurs from February to May, and fruits ripen in late June and early July. Fruits are capsules containing 30–50 small seeds with an elaiosome. Seeds are delivered when capsules prostrate on the ground after the bending of the pedicels, and open by five top-valves. Seeds are thought to be dispersed mainly by barochory, but there is evidence of occasional primary or secondary dispersal by ants, rodents and slugs (Jacquemyn et al. 2009; Valverde & Silvertown 1995; authors' unpublished data). Seeds are short-lived (Thompson, Bakker, & Bekker 1996) and there is no seed bank (Valverde & Silvertown 1995).

Experimental design

The experimental design was based on a Geographic Information System (GIS) of the study area (ArcGIS 9.1, Valdés & García 2009), which included data on forest cover and *P. vulgaris* cover. Forest cover was incorporated as a GIS layer by carefully digitizing each of the forest patches revealed by digital orthophotographies (scale 1:5000). A digitized layer of *P. vulgaris* cover was also incorporated in the GIS, created from schematic maps drawn during field surveys in the whole study area (see Valdés & García 2009 for details). We looked for locations showing contrasting environmental and population features resulting from gradients of habitats loss and fragmentation. In July 2009, we chose 6 study sites along the study area, representing sectors of high and low forest habitat amount (Fig. 1), each site comprised a pair of 50 m × 50 m plots 50–80 m apart. Plots in high forest cover sectors ranged from 73 to 100% forest cover, whereas those in low cover sectors ranged from 2 to 46% forest cover and included areas of fragmented forest interspersed with pastures and rocky areas. Plot pairs were chosen so that one plot contained natural *P. vulgaris* populations while the other did not. In each plot we established four sampling stations: two of them were located under dense forest canopy (canopy stations) and another two were located in pastures or canopy gaps (gap stations). Each of these stations comprised a set of three seed-sowing treatments (experimental replicates, each occupying an area of 10 cm × 10 cm where all standing vegetation was removed): *Exclusion* (20 seeds sown in two rows, covered with a square 4-mm mesh wire box, which was solidly fixed with nails in each of its corners, preventing access by seed predators and herbivores like rodents, birds, slugs, and carabid beetles), *Open* (20 seeds sown on an uncovered surface) and *Control* (no seeds added, to account for emergence from the potential natural seed bank). Seeds were collected from ripe fruits in a large number of *P. vulgaris* plants located all over the study area (seeds from different populations were mixed) and were sown immediately in order to keep their condition similar to how it would be after natural dispersal. All treatments were permanently marked.

We monitored all the experimental replicates on a monthly basis from the sowing till June 2010, in order to thoroughly follow seed germination in the first spring. After this date, surveys were made in August 2010, February 2011 and April 2011. The first seedling emergences were observed in March 2010. After that, we counted the number of emerged seedlings in every experimental replicate in each survey, following each plant individually and distinguishing new emergences from seedlings which had appeared earlier. In April 2011, we estimated plant size of all the remaining individuals. We estimated basal area by assuming an elliptical shape and measuring its major and minor diameters.

Data analysis

We considered three key survey dates for our analyses: May 2010 (to account for the cumulative germination by the end of the first spring), August 2010 (to account for first-summer mortality) and April 2011 (to account for first-winter mortality and possible second-spring germination).

We calculated response variables corresponding to seed germination, seedling survival and seedling growth separately for each experimental replicate. First, we constructed a binary variable called *emergence*, which represented the
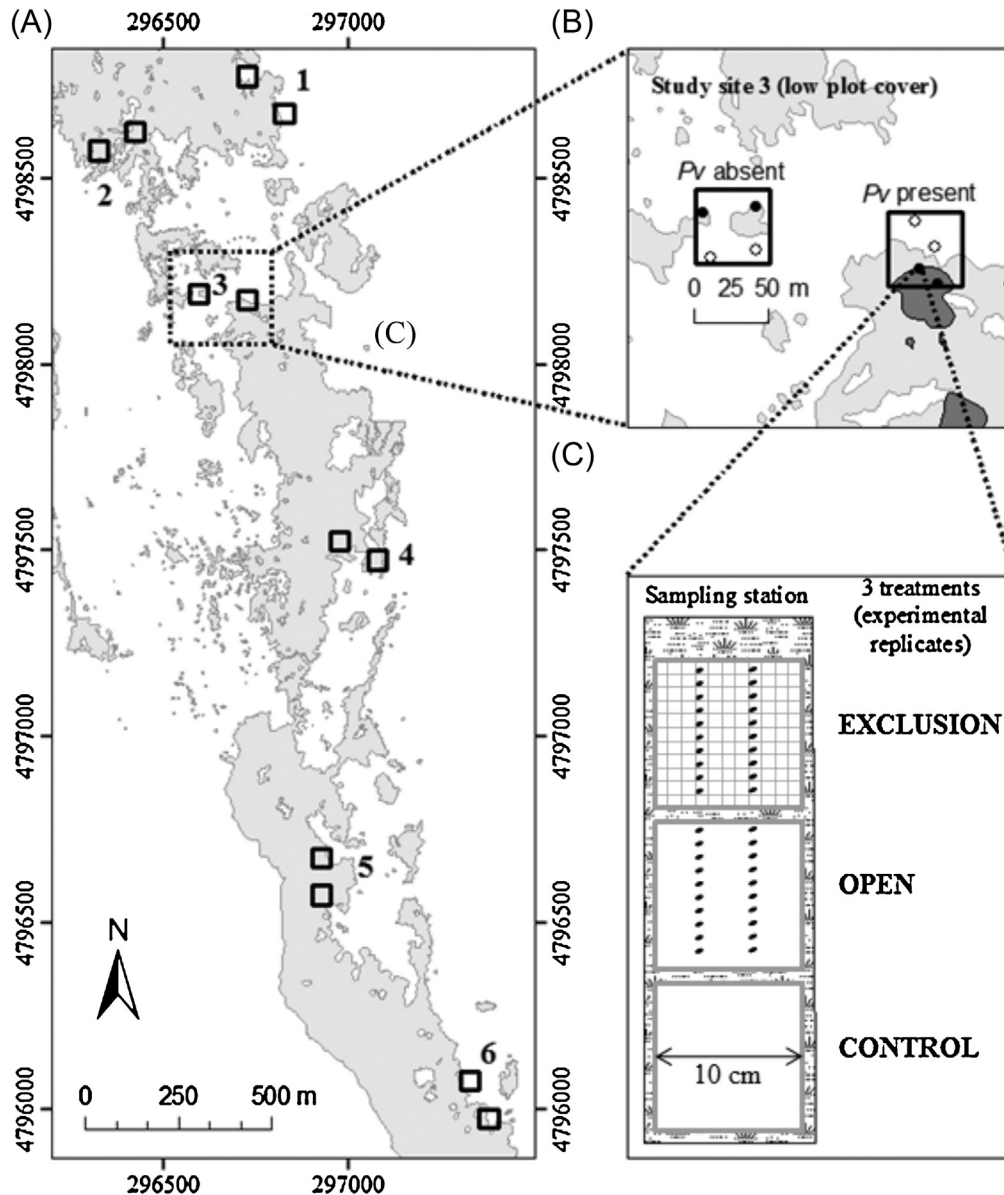
Fig. 1. Outline of the sampling framework. (A) Location of the six sampling sites along the study area. Forest cover is indicated by light gray shading. Sites 1, 3 and 6 contain low-cover plots, whereas sites 2, 4 and 5 contain high-cover plots. (B) Detail of one of the sites. The dark gray area represents *P. vulgaris* cover. Each site contains one 50 m × 50 m plot where *P. vulgaris* is absent and one where it is present. Each plot contains four sampling stations: two under canopy (black dots) and two in pastures or canopy gaps areas (white dots). (C) Station with 3 treatments (experimental replicates, see text for details).

occurrence of any seedling emergence until each of the three survey dates considered. Second, from August 2010 survey, we calculated the *survival after first summer* as the number of individuals counted at this survey date, divided by the total number of emergences until this date. The *survival after first winter* was calculated as the number of individuals counted during the April 2011 survey (excluding those newly emerged from August 2010 onwards) divided by the count in August 2010. Finally, the *size at one-year age* was calculated as the average size from all living individuals on April 2011.

We checked the response of the above variables in models incorporating the *plot cover* (as a measure of habitat amount,

i.e. forest cover, at the 50 m × 50 m plot scale, coded as low or high), the *presence* of the species in the 50 m × 50 m plot (in binary code with 1 = presence and 0 = absence), the *microhabitat* where the sampling station was located (canopy or gap), and the *experimental treatment* (exclusion, open, control). These models also included the second- and third-order interactions between predictors. Study site was not included as a predictor because previous analyses showed that variation between sites in all the response variables was negligible (results not shown).

We fitted Generalized Linear Models (GLMs) with R 2.13.0 (R Development Core Team, 2011) with different

Table 1. Summary of the GLM results. Only final models resulting from model selection are shown. Boldface indicates significance ($\alpha = 0.05$).

Response variables		Dependent variable	May 2010		August 2010		April 2011	
			Estimate	p	Estimate	p	Estimate	p
Emergence	*Control* included	Microhabitat (Gap)	0.17	0.7680				
		Treatment (*Control*)	−2.28	**0.0021**	−3.19	**<0.0001**	−3.39	**<0.0001**
		Treatment (*Exclusion*)	0.55	0.3672	1.16	**0.0309**	0.62	0.2207
		M (G) × T (C)	−1.36	0.3058				
		M (G) × T (E)	17.50	0.9895				
	Control removed	Microhabitat (Gap)	0.29	0.6280			−0.06	0.9319
		Treatment (*Exclusion*)	0.55	0.3670			$1.98\,e^{-16}$	1.0000
		M (G) × T (E)	17.39	0.9900			17.52	0.9899
Survival after first summer/winter[a]		Presence (1)			3.20	**0.0028**		
		Microhabitat (Gap)			1.63	0.1234	1.31	**0.0258**
		Treatment (*Exclusion*)			4.29	**0.0003**		
		Pr (1) × M (G)			−3.40	**0.0087**		
		Pr (1) × T (E)			5.89	**<0.0001**		
		M (G) × T (E)			3.83	**0.0046**		
		P (1) × M (G) × T (E)			5.88	**0.0005**		
Size at one-year age[a]		Presence (1)					0.51	0.3506
		Plot cover (Low)					1.15	**0.0054**
		Treatment (*Exclusion*)					2.36	**<0.0001**
		Pr (1) × Pc (L)					−3.17	**<0.0001**

[a] *Control* treatment removed, only experimental replicates with ≥1 seedling found at the survey date considered were included.

probability distributions and link functions depending on the response variable considered (see Table 1 for details). The choices of probability distributions for each model were made following Crawley (2007), in order to correct for potential overdispersion in the data. Separate binomial models (logic link) were fitted for emergence at each of the three survey dates and including all treatments, in order to check for dispersal limitation. These models were then repeated excluding the replicates corresponding to the *Control* treatment, in order to further explore the effect of the rest of the predictors in terms of establishment limitation between seed deposition and early emergence (e.g. due to seed predation). The models for survival after first summer and winter (quasibinomial, logit link), and that for size at one-year age (negative binomial, log link), were fitted including only those experimental replicates where at least one individual had been found at the survey date.

In all cases we fitted first a full model with the whole set of predictors and their second- and third-order interactions. We used stepwise combined model selection based on BIC (Bayesian Information criterion) to choose the most informative model, except in models with quasibinomial distribution (where BIC is not defined), in which model selection was made by successively removing non-significant factors from the full model.

Results

From the 1920 seeds sown, 414 seedlings (22%) emerged during the study period. More than 75% of these emergences happened before May 2010. Emergence from the natural seed rain was observed only in 8% of the experimental replicates assigned to the *Control* treatment, accounting for 54 seedlings (50 of which were found in the same experimental replicate). All of these emergences in the *Control* treatment occurred in plots where *P. vulgaris* was present.

The GLMs revealed that the probability of emergence differed between treatments in the three survey dates (Table 1), being lower in the *Control* treatment (9%, averaged for the three dates) than in the *Open* and *Exclusion* (70 and 87%, respectively). The *Control* treatment had a significant negative effect on emergence in the three survey dates, and in August 2010 the *Exclusion* had a significant positive effect. When the *Control* treatment was removed, all the predictors included in the model were not significant (and in August 2010 none of them was included in the model, Table 1).

Of all the emerged seedlings, 41% were still alive in August 2010, and 32% in April 2011. Survival responded differently to the set of predictors depending on the date considered (Table 1). After the first summer, there was a positive effect of *P. vulgaris* presence and of the *Exclusion* treatment, as well as significant interactions between these factors and also with the microhabitat (Table 1). This means that the effect of the experimental treatment on survival after the first summer depended on the microhabitat and on the presence of the species (Fig. 2). Exclusions of seed predators and herbivores increased significantly seedling survival in those replicates located far from adult populations and under canopy cover. On the other hand, survival after the first winter was only related to the microhabitat, being higher in pastures and canopy gaps (Table 1 and Fig. 3).
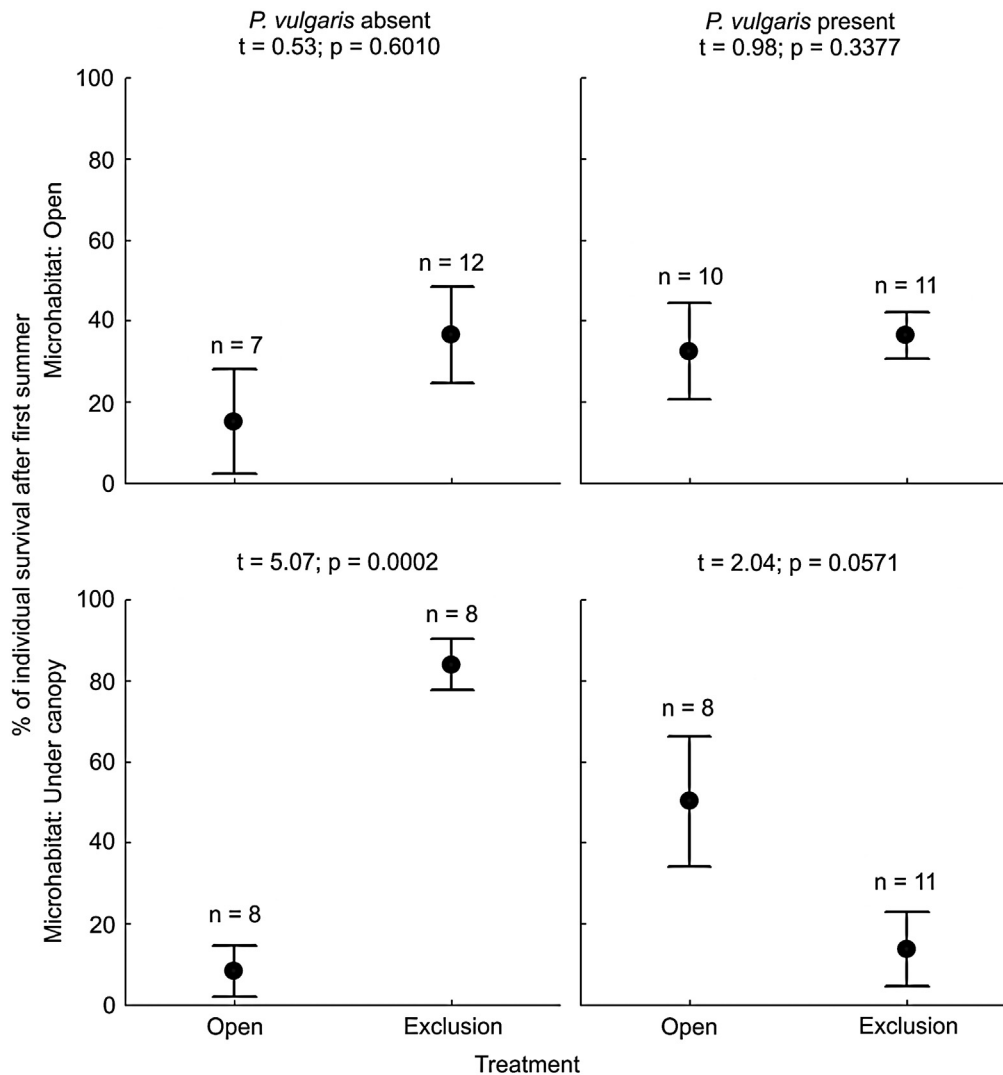
Fig. 2. Average % of individual survival (±SE) after first summer by treatment, plotted separately for each combination of *P. vulgaris* presence and microhabitat (*N*, *t*- and *p*-values resulting from a GLM fit in each case are shown).

When *P. vulgaris* was absent, the size of 1-year-old individuals was significantly larger in the *Exclusion* treatment and with low plot cover (Fig. 4).

Discussion

We experimentally assessed the mechanisms limiting early recruitment of *P. vulgaris* in a fragmented landscape, evidencing strong recruitment losses both at the stage of seed germination and emergence (78% of seeds did not germinate) and during early seedling life (32% of seedlings died within one year). We also evaluated how ecological gradients emerging from landscape alteration (i.e. those related with habitat amount, population size, and the presence of predators and herbivores) affected dispersal and establishment limitations. We found generalized dispersal limitation, regardless of the degree of landscape alteration. Establishment limitations were also independent of ecological gradients. Nevertheless,

habitat amount at the landscape and the microhabitat scale, population size and seedling predation affected both the survival and the growth of seedlings during the first year of life.

Dispersal limitation of seedling emergence

Our experiment suggests that *P. vulgaris* recruitment is highly restricted by seed availability, as very few emergences were observed in in experimental replicates where no seeds had been sown, even when reproductive adults were present nearby. These limitations seem to be related with the typically short dispersal distances of this species (no more than a few centimeters from maternal plants, Cahalan & Gliddon 1985; Campagne, Affre, Baumel, Roche, & Tatoni 2008) and the almost complete absence of animal-mediated seed dispersal. Accordingly, ants harvest only a small proportion of the seeds available (22% of seeds from seed depots after 15 days,
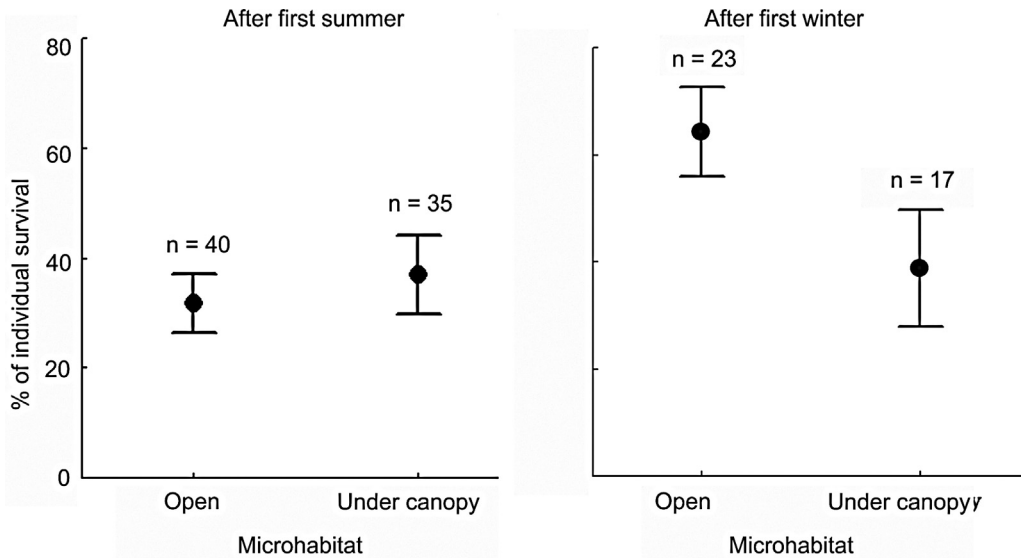
Fig. 3. Average % of individual survival (±SE) by microhabitat, plotted separately for survival after first summer and after first winter.

authors' unpublished data; see also Valverde & Silvertown 1995), and rodents seem to be ineffective in our study area, judging from the lack of a protective effect of the *Exclusion* treatment. Moreover, the fine-scale dispersal limitations evidenced here agree with the broad-scale dispersal limitation suggested by the strongly aggregated spatial distribution of *P. vulgaris* populations across the studied landscape (Valdés & García 2009).

None of the experimentally-controlled ecological gradients related to landscape alteration (habitat amount, population size, and the presence of predators and herbivores) affected the intensity of dispersal restrictions on seedling emergence. In fact, seedling emergence was only affected by seed availability, and not by seed predation. Germination rates were

equally low across all experimental gradients. This could be due to a generally low viability of sown seeds under field conditions, and it suggests also that germination drivers (e.g. soil humidity, temperature; Fay & Schultz 2009) operated at a much finer spatial scale than the one evaluated in our experimental design. Landscape alteration effects on *P. vulgaris* dispersal limitations seem to be caused by the decrease of seed production due to habitat loss, mostly derived from the flowering and pollination constraints of plants living in areas of low forest cover (Valdés & García 2011). Nevertheless, given the strong restrictions for seed movement even within habitat patches, we would expect negative effects of further fragmentation on recruitment as increased habitat isolation would even exacerbate dispersal limitation (long distance dispersal, although possible, e.g. Vellend, Myers,
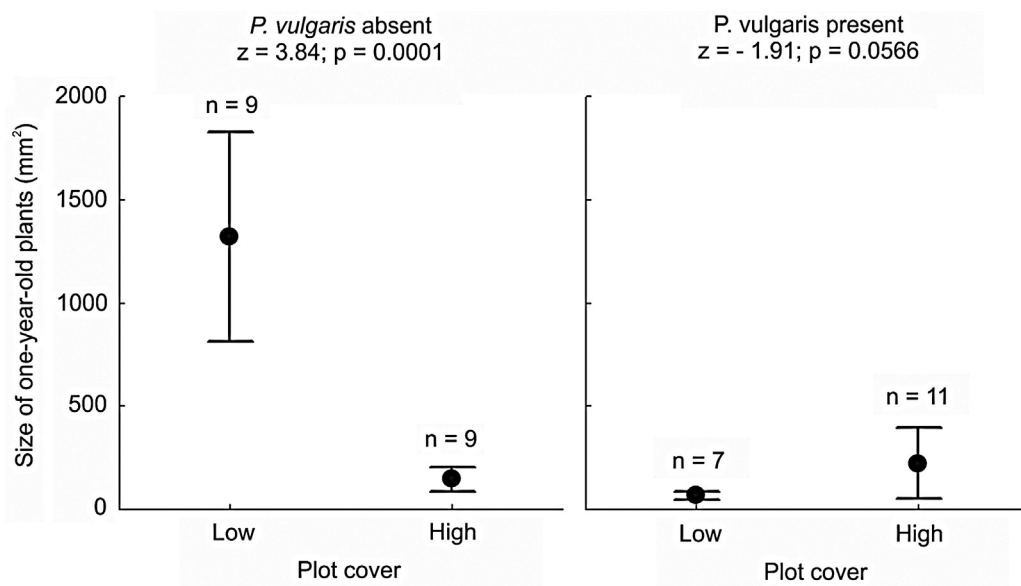

Fig. 4. Average size (±SE) in mm² of 1-year-old plants by plot cover, plotted separately for plots with natural *P. vulgaris* populations absent and present (z- and p-values resulting from a GLM fit in each case are shown).

Gardescu, & Marks 2003, seems particularly improbable in the study system, Valdés & García 2009).

Establishment limitation of seedling survival and growth

Our experimental gradients affected post-emergence seedling life, but these effects somehow differed from those expected from fragmentation theory (i.e. negative effects of increased habitat loss, population size decays or edge-promoted predator increase, Lindenmayer & Fischer 2006). Seedling survival after the first summer was similarly low across most of the tested factors, and only increased under specific environmental conditions. Namely, seedlings had a higher survival probability in those areas where many adult plants were already present. This suggests some kind of population-level habitat favorability (*sensu* Uriarte et al. 2010), which means that there is some underlying factor favoring both adult plant density and seedling survival. This factor could be, for example, soil humidity (Fay & Schultz 2009), as the effect was evident in summer but not in winter. Furthermore, survival of seedlings was also maximized in the *Exclusion* treatment, but this effect only occurred under tree canopies, and in areas where *P. vulgaris* was absent. This suggests that herbivore effect on survival was probably offset by other environmental factors in areas where *P. vulgaris* occurred, but in the areas lacking *P. vulgaris*, experimental seedlings were very conspicuous to natural enemies. Finally, the survival of seedlings after winter increased in pastures and canopy gaps, suggesting a beneficial effect of canopy removal, by reducing competition for light (Jurisch, Hahn, Wittig, & Bernhardt-Römermann 2013), which only emerged in the long term, as there was no effect of microhabitat after summer.

Seedlings reached bigger individual sizes when excluded from animals, revealing the direct effect of herbivory, or the indirect effect of a reduced competitive ability of animal damaged plants (Meiners & Handel 2000). Growth was also favored in sites with low amounts of forest habitat, probably derived from increased light inputs, but this response was stronger where *P. vulgaris* was absent, suggesting some intra-specific competition for light (Tyler & D'Antonio 1995; Wright 2002).

Concluding remarks

We have evaluated the effects of ecological gradients derived from habitat loss and fragmentation in *P. vulgaris* recruitment limitations in a fragmented landscape by using a seed-sowing experiment. As it has been stated that the bottleneck of recruitment occurs after seedling emergence (Ehrlén & Eriksson 2000), we tried to go beyond very short-term studies that look only at seed germination or seedling emergence by recording also further seedling survival and seedling size (as a surrogate of growth capacity), monitoring the plants for one year. We believe that the results obtained with this species could be extended to many species with similar characteristics and habitat requirements.

We confirmed the presence of both dispersal and establishment limitations. However, the recruitment of *P. vulgaris* is mainly restricted by dispersal limitation occurring at both local (within plots with *P. vulgaris* populations, as shown in this study) and broad scales (Valdés & García 2009), limiting local population size and plant distribution. Dispersal limitation and establishment limitations at the germination phase were not related to the ecological gradients studied and thus they do not seem to be dependent on landscape alteration. We acknowledge that seed germination could be more sensitive to small-scale variations in soil temperature, humidity, nutrient availability or microtopography not studied here. Nevertheless, habitat loss and fragmentation can condition survival and growth of seedlings by means of modifications of habitat amount at different scales, changes in plant population sizes and effects on seed predators and herbivores. This finding highlights the importance of extending the monitoring of seed-sowing experiments after the germination phase, as this allows the detection of barriers in the development of seedlings after their establishment. Some of the effects differed from those expected from classical fragmentation theory and from the responses observed in adults of the same species (e.g. higher seedling survival in open microhabitats, higher seedling growth in areas with low forest cover and absence of the species, see Valdés & García 2009, 2011 for responses in adult plants). This demonstrates that habitat requirements can differ between life stages and that there is a need of further seed-sowing experiments monitored till the adult stage in order to assess all the constraints that landscape alteration can impose during the whole plant life cycle.

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