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Effects of altitude, land use and microsites on early life performance of a high mountain tree: Insights from an in situ sowing experiment

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Abstract

Aim: Understanding the forces that drive range shifts in forest landscapes is imperative for predicting species distributions under anthropogenic climate and land use change. However, empirical studies exploring how these components jointly influence critical early life stages of mountain tree species across environmental gradients are scarce. We used the high mountain tree *Polylepis australis* as model species to investigate the relative importance of altitude and associated climatic conditions, land use for livestock and microsite characteristics on early life performance.

Location: Córdoba Sierras, central Argentina.

Methods: We set up an extensive in situ sowing experiment with a robust split-plot design that integrated spatial scales ranging from 0.4 m^2 subplots at the microsite level (associated with vegetative and microtopographic structures), to livestock exclosure and enclosure plots of several hectares, to an altitudinal gradient of 1,000 m. Components of early life performance were monitored across two subsequent growing seasons.

Results: Microsite characteristics played a fundamental role in *P. australis* establishment, whereby interactions with altitude and/or land use suggested alternate mechanisms: facilitation (likely reduced desiccation) dominated at low altitude while at high altitude, abiotic stress (likely intensive frost and radiation) overruled any microsite effects. At mid-altitude, benefits of competition release prevailed over facilitation and microsite effects gained importance under livestock presence. Inconsistencies between pre- and post-emergence responses illustrated potential trade-offs between beneficial and detrimental effects of microsite conditions upon performance throughout early life: a favourable location for seeds may abruptly turn adverse for seedlings.

Main conclusions: We unravel how changes in altitude, anthropogenic disturbances and microsite characteristics jointly modulate *P. australis* performance across stages of early establishment. Such information is fundamental when categorizing specific

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microhabitats as "safe sites" for tree regeneration especially in mountain environments with high spatio-temporal heterogeneity.

KEYWORDS
abiotic factors, competition, facilitation, livestock, microsites, *Polylepis australis*, recruitment, safe sites, seed regeneration, seedlings

1 | INTRODUCTION

Understanding the forces that shape establishment of plant species is highly important for the prediction of their future distribution in the face of global change. Considerable attention has been drawn to mountain forests, which are currently under tremendous pressure from global change (Beniston, 2003; Bonan, 2008; FAO, 2016). Mountain forests make up a third of all natural forest cover worldwide, and many of their tree species play key roles in providing habitat, food or mutualistic benefits to numerous species of animals, fungi, micro-organisms and other plants (Price et al., 2011). However, research-based management strategies for effective restoration and conservation of mountain forests are missing for many regions.

Changes in precipitation, temperature regimes as well as land use intensification have altered species interactions and microsite quality/availability with critical consequences for regeneration vital rates of tree species (reviewed in Hansen et al., 2001), ultimately driving community reshuffling, biodiversity loss and possibly forest death (Walther, 2003, Parmesan, 2006).

Decreased germination rates and recruitment are considered to be primary mechanisms forcing changes in distribution and driving plant populations to local extinction (Kitajima & Fenner, 2000). Seedling establishment at a given site is therefore a useful metric for the effects of climate, land use and microsite conditions on plant species at their range limits (Louthan, Doak, & Angert, 2015). A rich body of literature offers insights on effects of those environmental drivers in isolation while very few conclusive experimental tests have studied their interactive effects on early life cycle stages.

Early life performance of mountain tree species has been assessed along altitudinal gradients as proxy for several environmental variables (namely, temperature, UV exposure and precipitation). These studies have, for example, identified low temperatures (Maher & Germino, 2006; Piper, Cavieres, Reyes-Díaz, & Corcuera, 2006; Rada, García-Nuñez, & Rangel, 2011), intense solar radiation and desiccation (Bader, Van Geloof, & Rietkerk, 2007; Gieger & Leuschner, 2004), soil properties (Davis, Hager, & Gedalof, 2018) as crucial determinants of germination, seedling performance and survival. Investigating the effect of altitude on seedling performance has also provided evidence for genetic differentiation (Marcora, Tecco, Zeballos, & Hensen, 2017) as well as for species-specific regeneration niches (Morales, 2017). Anthropogenic influence, mainly through land use for livestock rearing, has shown to have diverse impacts. Descriptive studies report detrimental effects on seed production and viability by direct browsing or indirect soil degradation (Pollice, Marcora, & Renison, 2013; Renison, Hensen, & Cingolani, 2004), whereas negative effects on seedling recruitment and survival have been reported from experimental settings (Gill & Beardall, 2001; Speed, Austrheim, Hester, & Mysterud, 2010). Contrarily, other studies have remarked positive effects of moderate livestock disturbance on seedling densities inside forests and across the mountain forest ecotone (Darabant, Rai, Tenzin, Roder, & Gratzer, 2007; Zimmermann, Renison, Leyer, & Hensen, 2009) because browsing and trampling may also reduce existing vegetation cover and thereby increase soil temperatures that facilitate germination and seedling emergence. These apparently contradicting results suggest a complex context-dependent influence of land use for livestock rearing on forest regeneration (Cairns & Moen, 2004). The specific functional role of vegetative and microtopographic facilitation involved in early life stages has only recently been considered as an important mechanism determining safe site for regeneration in trees (Llambí, Puentes Aguilar, & García-Núñez, 2013; Maher & Germino, 2006; Smith, Germino, Johnson, & Reinhardt, 2009). On the one hand, plants or abiotic physical structures in immediate vicinity can facilitate recruitment by sheltering seedlings from adverse climatic effects and herbivores (Batllori, Camarero, Ninot, & Gutiérrez, 2009; Carlucci, Duarte, & Pillar, 2011; D'Odorico et al., 2013). On the other hand, a dense vegetation or cover of abiotic structures can impede seedling emergence and establishment by limiting resources such as light, water, nutrients and access to suitable seedbed (Moir, Rochelle, & Schoettle, 1999; Thrippleton, Bugmann, Kramer-Priewasser, & Snell, 2016).

Establishment bottlenecks clearly occur in early life stages, yet analysing the relative importance and the interactions between biotic and abiotic key factors remains a scientific challenge: theory suggests that abiotic constraints primarily determine vital rates in harsh environments (e.g. at high latitudes or altitudes) and biotic interactions do so in benign environments (e.g. at low latitudes or altitudes), reviewed in Louthan et al. (2015). Moreover, facilitative effects are predicted to be more common under harsh abiotic or disturbed conditions, whereas antagonistic interactions prevail in benign habitats (sensu Callaway et al., 2002). Nonetheless, environmental factors rarely vary in parallel along gradients (e.g. altitudinal) conditioning the outcome of the competition–facilitation trajectory. Furthermore, livestock rearing may alter resource availability by defecation, create small-scale physical disturbances by trampling and relax competition according to animals' foraging preferences (Mazzini, Relva, & Malizia, 2018; Mortensen et al., 2017). Finally, the effects of microsite conditions can be highly complex and the

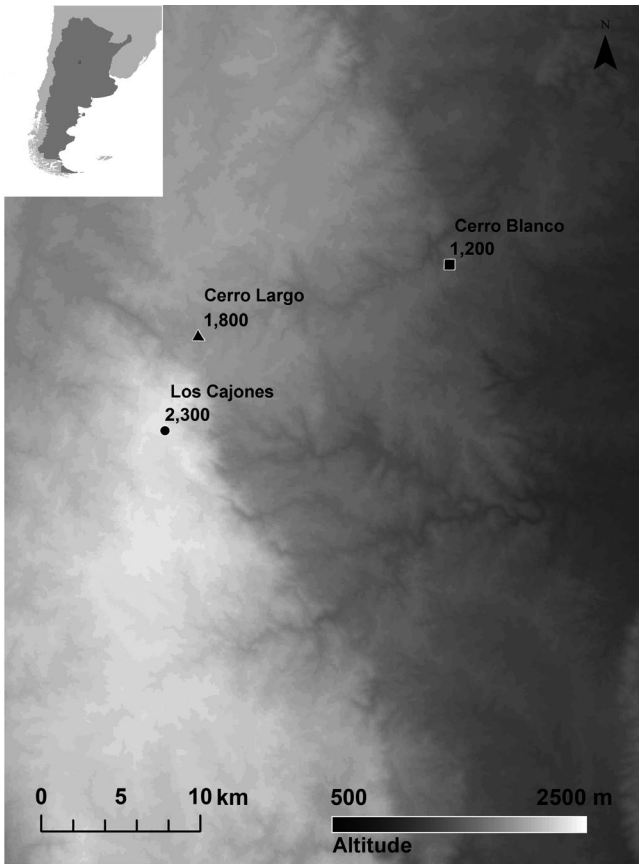


FIGURE 1 Locations of experimental sites along the studied altitudinal gradient in the mountain range of Sierras de Córdoba, central Argentina. Cerro Blanco at 1,300 m (■ S 31°20.687′ W 064°39.194′), Cerro Largo at 1,800 m (▲ S 31°23.097′ W 064°47.732′) and Los Cajones at 2,300 m (● S 31°26.318′ W 064°48.854′)

suitability of particular microsites for regeneration not only depends on their very nature (i.e. nursing structures or nursing plants; Haussmann, McGeoch, & Boelhouwers, 2010; Munguía-Rosas & Sosa, 2008) but also on the particular climatic stressors existing along altitudinal gradients, on the type of prevailing human land use activities and the spatial configuration of vegetation and microtopography. Consequently, there is an apparent need for studies that address potential interactions among climate, land use and fine-scale microsite characteristics in order to gain an improved understanding of recruitment limitation and regeneration dynamics in mountain forests and to comprehensively inform management decisions.

Polylepis mountain forests of South America are widely distributed along the Andean and adjacent mountain ranges with individual trees reaching elevations as high as 5,000 m. Similar to other mountain forests, they provide fundamental source of habitat for a wide range of associated endemic species and valuable ecosystem services while they continue to be threatened by habitat loss, fragmentation, and degradation and face future threats from climate change (Gareca, Hermy, Fjeldså, & Honnay, 2010; IUCN, 2017). The results of previous work (Morales, Sevillano-Rios, Fick, & Young, 2018; Rehm & Feeley, 2015; Renison, Morales, Cuyckens, Sevillano, & Amaya, 2018) suggest this system is both excellently suited and of highest priority to investigate mechanisms controlling recruitment of mountain tree species. Today, it is acknowledged that the current distribution of *Polylepis* forests largely reflects a combination of climatic and land use influences but the concrete nature of these effects has been rarely assessed, in particular with a focus on seed regeneration.

To our knowledge, no previous seed-addition study has evaluated changes in early life performance and simultaneously assessed the relative importance of altitude, land use and microsite conditions. Experiments that comprehensively test for recruitment limitation remain rare because their inherent labour intensiveness, high seedling mortality and the difficulty of manipulating many environmental factors presumed to influence tree recruitment under field conditions. Using *P. australis* as model species, we present an in situ sowing experiment that aims to investigate the individual and combined effects of altitude and associated climatic conditions (low [dry/warm] vs. mid [moderate precipitation/temperature] vs. high [wet/cold]), land use (livestock absent vs. present), microsite type (rocks vs. tussocks vs. pastures vs. bare soil) on several components of early life performance in the field. We hypothesized that (a) seedling emergence, seedling performance, recruitment and survival are lower at low and high altitudes, commonly associated with relatively harsher warm/dry and wet/cold conditions, respectively, and that these negative effects are amplified by additional exposure to livestock presence; (b) microsites associated with rocks or tussocks provide freezing (high altitude) or desiccation (low altitude) protection and thus facilitate higher early life performance than microsites in pasture and bare soil; (c) the negative effects of less suitable microsite types on components of early life performance are exacerbated under the presence of livestock at low and high altitudes.

2 | METHODS

2.1 | Study species

The endemic treeline species *Polylepis australis* (Rosaceae) is the southernmost species of this genus and dominates the canopy of open-canopy forests (also called woodlands). In central Argentina, *P. australis* individuals can be found from 900 to 2,700 m.a.s.l., with highest performance at around 1,800 m according to growth and seed production (Marcora, Hensen, Renison, Seltmann, & Wesche, 2008) and around 1,900 m according to *P. australis* forest cover, post-fire survival and growth (Argibay & Renison, 2018). The species is wind pollinated and self-incompatible (Seltmann, Cocucci, Renison, Cierjacks, & Hensen, 2009), and fruits are single-seeded nutlets (hereafter referred to as seeds) that ripen and fall from the trees between January and April. They usually germinate immediately after wind dispersal at the end of the wet season in March and April. Seed viability is variable, ranging from 0% to 60% with an average viability of around 20% (Renison et al., 2004).

2.2 | Study area

Our experiment was conducted at the mountain range of Sierras de Córdoba in central Argentina (31°25′S; 64°37′W; 500–2,800 m; Figure 1). Along the studied gradient, several climate variables are correlated with altitude. Mean annual temperature decreases from 15.7°C at 900 m to 7.4°C at 2,700 m (Marcora et al., 2008), and annual precipitation increases from 600 to 1,000 mm between 600 and 2,200 m (Colladon, 2014). Moreover, soil temperature decreases and soil moisture increases towards the upper portions of the gradient (Pais-Bosch, Tecco, Funes, & Cabido, 2012; Tecco et al., 2016). Dry–cold season occurs from May to September, while most of the rainfall is concentrated in the warmer months between October and April. The soils are derived from weathering of the granite substrate and fine-textured Aeolian deposits (Cabido, Breimer, & Vega, 1987).

The landscape consists of a mosaic of rocky outcrops, tussock patches, pasture lawns and open soil patches surrounding the scattered distributed mountain forest relicts and shrublands. The canopy of the forest–shrubland patches is dominated by *P. australis* together with other less abundant woody species such as the tree *Maytenus boaria* Molina (Celastraceae) and the tall endemic shrub *Escallonia cordobensis* (Kuntze) Hosseus (Escalloniaceae; Renison et al., 2015). Land use for livestock rearing in the area (mainly cattle and horses) dates back to the 17th century and has produced extensive modification of the vegetation and huge areas with soil loss (Cingolani et al., 2013).

2.3 | Seed collection and preparation

Seeds were hand-harvested in February and March 2016 from 10 to 15 individuals in 11 accessible forest fragments. Fragments were distributed along altitudes ranging from 1,542 to 2,100 m and located at maximum distance of 70 km from our experimental sowing sites (Table S1). Following Pérez-Harguindeguy et al. (2013), on each sampling location, seeds were collected from vigorous-looking, sexually mature, haphazardly selected individuals. In the laboratory, collected seeds were cleaned and pooled. Afterwards, average mass of twenty randomly selected subsamples each of 300 seeds (2.347 ± 0.026 g) was obtained using a precision balance (ENTRIS 124i-1S, Sartorius Lab Instruments, Göttingen - Germany). Based on this average mass, the 300 seeds of each seed batch added to subplots (see below) were gravimetrically determined rather than counted. Each seed batch was separately stored in small paper bags at room temperature until the start of the experiment. Twenty seed batches were randomly selected and used to verify the number of seeds per batch (300 ± 5 seeds).

2.4 | In situ sowing experiment

The experiment was carried out along an altitudinal gradient in a sector known as “Los Gigantes” at three altitudes: Cerro Blanco at 1,300 m, Cerro Largo at 1,800 m and Los Cajones at 2,300 m (hereafter, low, mid- and high altitudes, respectively). At each altitude, we established two randomly selected experimental sites at a distance of 100–500 m from each other, in order to cover varying habitat conditions. Each site consisted of two main plots, one plot fenced (livestock absent) and the other one located outside the exclosure (livestock present). Within each site, we established 30 blocks located inside the livestock exclosure and another 30 blocks located outside the livestock exclosure with a minimum distance between blocks of 10 m. Each block contained four subplots of 0.2 m × 0.2 m randomly assigned to one out of four microsites: rocks (R), tussock grasses (T), pasture lawns (P) or bare soil (S). Each subplot was marked with a numbered metal pin of 40 cm length fixed to the soil; additional GPS coordinates were recorded to facilitate retrieval of subplots. In order to minimize the possible confounding effects of larger-scale environmental heterogeneity, blocks were constrained to those places where all of the four microsite types were present within a 3 m radius. When having multiple choices for a given microsite type within a block (mostly for rocks and tussocks), then microsite selection was done randomly.

Rock microsites comprised any kind of microhabitat delimited laterally (<10 cm apart from the rock edge) by a rock outcrop. Tussock microsites were located upslope and adjacent (<10 cm) to a discrete patch of tussock grass (*Festuca hieronymi* Hack, *Jarava pseudoichu* (Caro) F. Rojas, *Nassella filiculmis* (Delile) Barkworth and *Aristida laevis* (Nees) Kunthat 1,300 m, *Festuca hieronymi*, *Deyeuxia hieronymi* (Hack.) Türpe at 1,800 m and *Poa stuckertii* (Hack.) Parodi at 2,300 m; all Poaceae species). Pasture lawn microsites harboured different species of short growing grasses and forbs. Vegetation on tussock and rock microsites was similar to pasture lawns but in most cases, it also included lichens and mosses, which were virtually absent from tussock and pasture microsites. Bare soil microsites were artificially created on a 50 cm × 50 cm area using a small shovel, by superficially clearing above-ground vegetation but leaving roots and non-organic material in place (Aarssen & Epp, 1990; Tingstad, Olsen, Klanderud, Vandvik, & Ohlson, 2015). Since vegetation regrew within several weeks at mid-altitude, we had to clear the subplots more than once at these sites. In contrast, we did not observe significant regrowth during the whole experimental period at low and high altitudes.

In summary, our experiment was set up according to a split-plot design (see Figure 2). The main plots representing different livestock treatments (exclosure vs. livestock present) were considered as the main plot level, and the subplots representing the different microsites were considered as the subplot level. Furthermore, our experiment included two nested blocking factors; first, the two sites nested within altitude and second, the 30 blocks nested within each main plot. This design resulted in a total of 1,440 subplots (3 altitudes × 2 sites × 2 livestock treatments × 30 blocks × 4 microsites).

2.5 | Livestock treatment and microclimatic characterizations at each altitude

High, mid- and low altitudes were fenced in 2012, 2013 and 2015, respectively. Assuming that community responses become slower

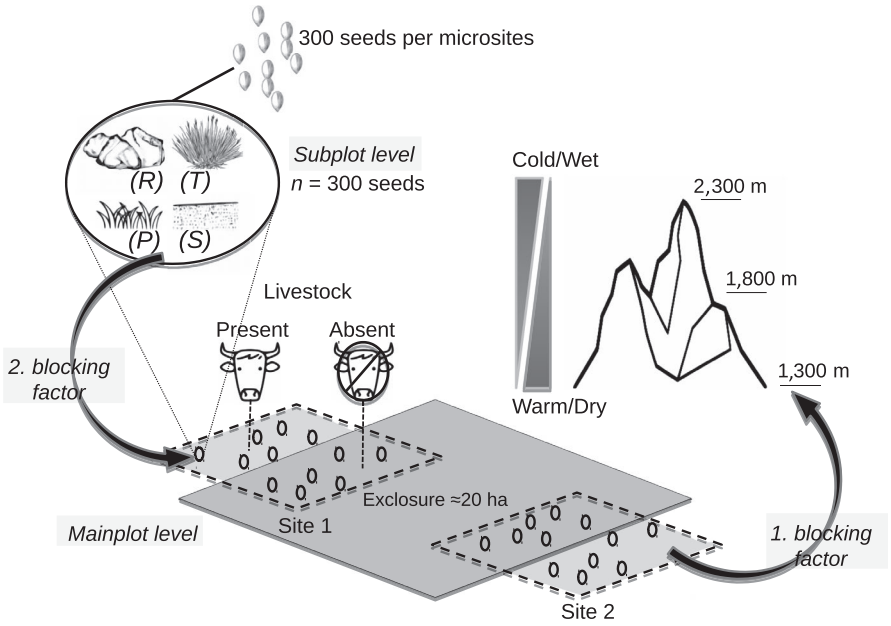


FIGURE 2 Schematic representation of the experimental design: at each altitudinal level (low: 1,300 m, mid: 1,800 m, high: 2,300 m), in situ sowings were performed under livestock absence and presence at two randomly selected sites (main plot level). Thirty randomly assigned blocks, each of them containing four different microsites (subplot level)—rocks (R), tussock grasses (T), pasture lawns (P) and bare soil (S)—were established inside and outside the livestock exclosure. The altitudinal gradient is characterized by a steep change in temperature and in precipitation

with increasing altitude, higher altitude exclosures had more time to change than lower altitude exclosure, compensating for the differences in change rate at least in part. We constructed the fences large enough (total fenced area of ≈20 ha) to cover both exclosures, except for the lower site where, for logistic reasons, two separate livestock exclosures (1 ha each) were built. Given the size of the fenced area and the distances between the two sites, we still consider them independent. Livestock density was constant throughout the experiment and similar between altitudes, ranging from 0.2 to 0.4 cattle equivalents per hectare (CE/ha) as estimated from dung counts performed once a year using the model developed for the study area (see von Müller, Cingolani, Vaieretti, & Renison, 2012). These values are comparable to those reported within the entire mountain system of Sierras de Córdoba and considered low to moderate (Renison et al., 2015; Teich, Cingolani, Renison, Hensen, & Giorgis, 2005; Vaieretti, Cingolani, Harguindeguy, Gurvich, & Cabido, 2010).

In addition, to characterize general microclimatic conditions across altitude, recordings at each altitudinal level were done by means of a HOBO H21-002 Micro Station (Onset Computer Corporation) installed during the first fieldwork season from February to July 2016 (see full summary in Table S2). Furthermore, to evidence how daily courses of microclimatic conditions can differ among microsites as well as along the altitudinal gradient, microclimatic recordings for one single subplot (1 data logger per microsite), on each altitude, were performed at soil surface every 10 min. To this end, iButtons (DS1923-F5# Maxim integrated) were set up during the second fieldwork season from January to February 2017 (Figure S3).

2.6 | Seed additions and early life performance measurements

Seeds were intentionally added between the 3rd (high altitude) and 4th week of March (mid- and low altitudes) 2016, to simulate a late natural seed rain. Specifically, we added 300 *P. australis* seeds to each subplot resulting in a total of 432.000 seeds. Maximum seed dispersal distance in *P. australis* is reported to be <10 m (Torres, Renison, Hensen, Suarez, & Enrico, 2008); accordingly, natural seedling recruitment in *P. australis* is limited to the close neighbourhood of seed-producing adults. Renison et al. (2015) controlled for natural seed dispersal in a study that employed similar methods in this area; considering a total 252 control plots, they detected a total of only one seedling which was at a site near a seed-producing adult. In our experiment, there were no adult trees within a 100 m radius around our plots, and we found no seedling outside our seed-addition plots when inspecting the experimental sites. We thus consider natural seed input to be negligible in our experiment, with no need to control for it. Furthermore, seed predation is likely to be low as *Polylepis* fruits are woody and we could not find in the literature any reports of predation by birds or rodents, while predation by insects is reported to be <1% (323 out of 34,100 evaluated seeds, Lett, 2006).

During the first growing season after sowing (May 2016, hereafter termed t1), we carefully searched for seedlings on each microsite subplot and recorded the total number present (hereafter termed seedling emergence). Up to ten randomly chosen seedlings were marked with metallic sewing pins. We recorded seedling height and the number of true leaves of the marked individuals and calculated the mean for both variables per subplot. Moreover, we determined the proportion of marked individuals exhibiting reddened leaves, which we considered as a symptom of physiological stress (Chalker-Scott, 2002; Gould, 2004). During the second growing season after sowing (March 2017, hereafter termed t2), we again determined the total number of seedlings present within each subplot (hereafter termed seedling recruitment) which included recently emerged seedlings (cotyledons present) and the proportion of previously marked individuals that

TABLE 1 Results of generalized linear mixed-effects models on the main and interactive effects of altitude (Alt), livestock (LS) and microsite (MS) on seedling emergence, recruitment, survival and seedling performance of *Polylepis australis*

	First growing Season (t1)				Second growing Season (t2)				
	Seedling emergence (proportion)	Seedling height (cm)	Number of leaves	Stressed seedlings (proportion)	Recruitment (proportion)	Survival (proportion)	Seedling height (cm)	Number of leaves	Stressed seedlings (proportion)
Altitude (Alt)	**$p < 0.0001$**	**$p < 0.0001$**	**$p = 0.0003$**	**$p = 0.0019$**	**$p = 0.0134$**	**$p = 0.0939$**	**$p < 0.0001$**	$p = 0.1572$	$p = 0.709$
Livestock (LS)	$p = 0.5377$	**$p = 0.017$**	$p = 0.1359$	$p = 0.0909$	$p = 0.1507$	$p = 0.2052$	$p = 0.4088$	**$p = 0.0045$**	**$p = 0.0074$**
Microsite (MS)	**$p < 0.0001$**	**$p < 0.0001$**	$p = 0.1138$	**$p < 0.0001$**	**$p = 0.0242$**	**$p < 0.0001$**	**$p = 0.0032$**	**$p < 0.0001$**	**$p < 0.0001$**
Alt × LS	$p = 0.7925$	$p = 0.1126$	$p = 0.4192$	$p = 0.5064$	$p = 0.9318$	$p = 0.8879$	$p = 0.4244$	$p = 0.1778$	$p = 0.3356$
Alt × MS	**$p < 0.0001$**	**$p < 0.0001$**	**$p < 0.0001$**	**$p = 0.0374$**	**$p < 0.0001$**	**$p < 0.0001$**	**$p = 0.0003$**	$p = 0.0697$	$p = 0.1336$
LS × MS	$p = 0.2804$	**$p = 0.0003$**	$p = 0.2094$	$p = 0.1166$	$p = 0.2379$	$p = 0.5987$	$p = 0.3965$	$p = 0.0831$	$p = 0.0914$
Alt × LS × MS	$p = 0.3545$	$p = 0.881$	$p = 0.4417$	**$p = 0.0104$**	$p = 0.4473$	$p = 0.8159$	$p = 0.7254$	$p = 0.4229$	$p = 0.1716$

Note: The table shows *p* values for the respective fixed effects in the full model (bold values indicate significance at $p < 0.05$).

had survived within each subplot. For previously marked individuals, we recorded the same data as described for the first growing season and calculated mean values per subplot based on remaining individuals. Seedling height was measured from the base to the cotyledon tip (or highest true leaf tip). Hereafter, when referring to all measured components of early life performance, we distinguish two points in time: seedling emergence and associated plant performance at t1; and recruitment, survival and associated plant performance at t2.

2.7 | Data analysis

All data were analysed using SAS version 9.4 (Procedure GLIMMIX). Microsite subplot was the observation unit in all analyses. For responses with normal error distribution (mean seedling height t1 and t2, mean number of leaves t1 and t2), we used linear mixed-effects models (LMMs), whereas for responses with binomial error distribution (seedling emergence t1, recruitment t2, survival t2, proportion stressed seedlings t1 and t2), we used generalized linear mixed-effects models (GLMMs). The GLMMs for seedling emergence at t1, stressed seedlings at t1 and t2 and recruitment and survival at t2 were fitted with a binomial error distribution and logit link function. A multiplicative overdispersion parameter was added to account for overdispersion if necessary. The models for all response variables comprised the fixed factor altitude (low, mid, high), livestock (absent, present) and microsite (rock, tussock, pasture, soil) as well as all possible interactions among them. According to the split-plot design of the experiment, all models contained the random effects of site nested within altitude, livestock treatment x site nested within altitude (the main plot error) and block nested in livestock treatment x site x altitude. The models for seedling emergence at t1, mean seedling height at t1, mean number of leaves at t1, stressed seedlings at t1 and recruitment at t2 were calculated for the full dataset (1,440 plots). The model for mean seedling height at t2 was calculated on a data set that excluded S microsites at high altitudes because the number of seedlings was too low on this particular microsite. For survival at t2, mean number of leaves at t2 and stressed seedlings at t2 models included only low- and mid-altitude data sets (960 plots), as the majority of marked seedlings had died at t2 at the high altitude.

To test significance of fixed effects, we calculated chi-square tests for each main and interaction effect with all the other effects in the model (i.e. type III tests of fixed effects; for parameter estimates tables resulting from all fitted models see Tables S4–S5). For illustration of the combined effects of altitude, livestock and microsite on the response variables, we extracted least square means (LS-means) with standard errors from the full models. In contrast to raw data means and their standard errors, these model estimates account for the specific error distribution of the responses as well as for all other fixed and random effects in the model. In cases of significant main or interaction effects, a-posteriori comparisons among the respective LS-means were performed using Tukey tests (see visual summary in Figure S6).

3 | RESULTS

3.1 | Seedling emergence and seedling performance in the first growing season (t1)

Two months after in situ sowing took place, we recorded a total number of 8,641 emerged seedlings in the 1,440 subplots established across the altitudinal gradient. In general, emergence events observed during the first growing season decreased in frequency towards high altitude (low: 5,399, mid: 2,905, high: 337).

Microsite effects on seedling emergence significantly differed among altitudes. Differences observed between microsites were less prominent at mid compared with high and low altitudes (significant interaction altitude × microsite; Table 1). Specifically, at low altitude, highest emergence occurred in tussock, intermediate in rock and pasture microsites, while lowest in bare soil microsites. At mid-altitude, no significant differences between microsites were found. At high altitude, lowest emergence occurred in bare soil microsites, whereas in all three other microsite types, it was consistently higher (Figure 3a; Figure S6a).

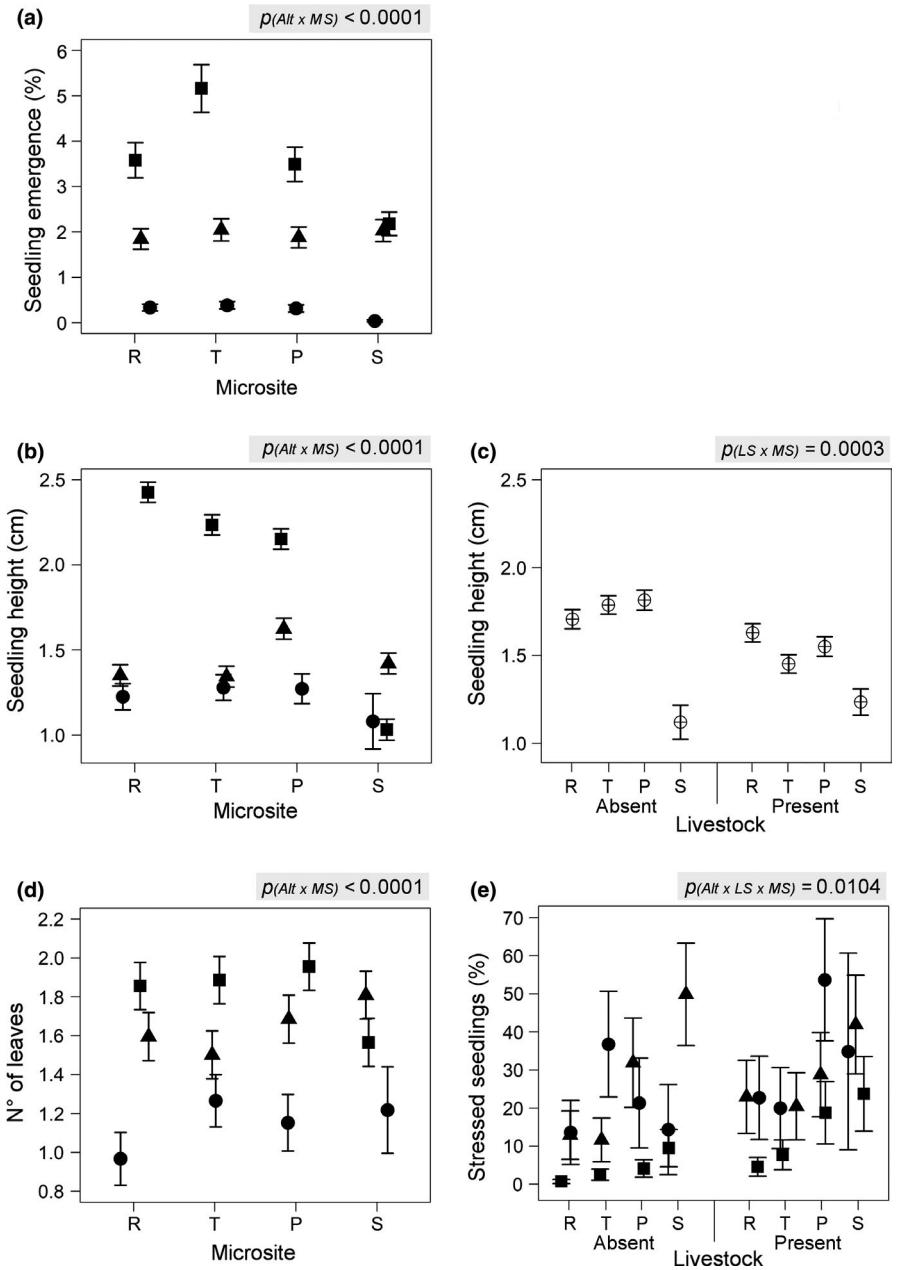


FIGURE 3 Effects of altitude (low: ■, mid: ▲, high: ●), livestock (absent: left hand, present: right hand) and microsite (rock [R], tussock [T], pasture [P], bare soil [S]) on (a) seedling emergence, (b, c) seedling height, (d) number of leaves and (e) proportion of stressed seedlings during the first growing season (t1). The symbols and bars represent the least square means and standard errors predicted by the full models. Levels of significance for the interactions shown are denoted on top of each plot. For each model, we plotted the highest order significant interactions

Seedling height variation among microsites tended to be less pronounced towards high altitude (significant interaction altitude × microsite; Table 1). At low altitude, tallest seedlings were found in rock microsites, intermediate sized seedlings were found in tussock and pasture microsites, while smallest seedlings were found in bare soil microsites. At mid-altitude, only pasture showed significantly higher seedlings than rock, tussock and bare soil microsites, which showed no significant differences between them. At high altitude, no significant differences in seedling height between microsites were found (Figure 3b; Figure S6b). Livestock presence significantly decreased seedling height only in tussock and pasture microsites (significant interaction livestock x microsite; Table 1). Moreover, under livestock absence, smallest seedlings were consistently found only in bare soil microsites, with no significant differences among the rest of microsites. Under livestock presence, seedlings in rock microsites were significantly taller than seedlings in tussock and bare soil microsites (Figure 3c, Figure S6c). Irrespective of livestock presence, the number of leaves generally decreased with altitude yet this effect differed among microsites (significant interaction altitude x microsite; Table 1). At low altitude, leaf production was lowest in bare soil with no differences among the remaining microsites. At mid-altitude, the number of leaves was only significantly lower in tussock microsites as compared to bare soil, while microsite differences were again nonsignificant at high altitude (Figure 3d; Figure S6d). Combined effects of altitude, livestock and microsite influenced the proportion of stressed seedlings (significant interaction altitude × livestock × microsite; Table 1). At low altitude, livestock presence accentuated microsite suitability differences (absent: R < S, T < S; present: R < P, R < S, T < P, T < S) while under moderate conditions at mid-altitude, microsite suitability differences were fewer (absent: R < P, R < S, T < P, T < S; present: R < S, T < S). Finally, regardless of livestock presence, at high altitude, we did not find evidence of differences between microsites (Figure 3e; Figure S6e).

3.2 | Recruitment, survival and seedling performance in the second growing season (t2)

We recorded a total number of 1,407 recruited seedlings, including 118 newly emerged seedlings and 1,163 previous-year surviving seedlings. The number of recruited seedlings was highest at mid-altitude (low: 575, mid: 683, high: 149), which was also the case for number of surviving seedlings (low: 498, mid: 601, high: 64). In the time course between t1 and t2, seedlings grew approximately 1.7 (low); 1.5 (mid) and 1.2 (high) times bigger. New emergence events observed during the second growing season appeared to increase in frequency towards high altitude (low: 6, mid: 28, high: 84).

Microsite differences affected seedling recruitment to a stronger extent at mid and low than at high altitude (significant interaction altitude × microsite; Table 1). At low altitude, lowest seedling recruitment was consistently found in pasture microsites, with no significant differences among the rest of microsites. At mid-altitude, highest recruitment was found to occur in bare soil microsites, whereas at high altitude, no significant differences among microsites were detected (Figure 4a; Figure S6f). Microsite effects on seedling survival also differed between altitudes (significant interaction altitude × microsite; Table 1). At low altitude, lowest seedling survival

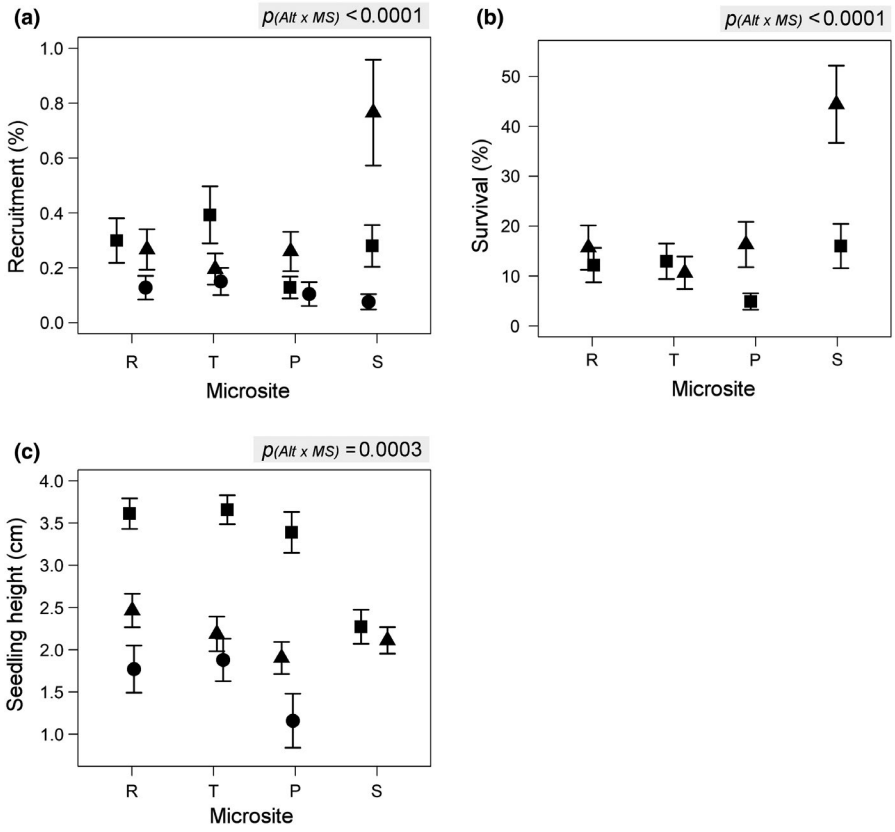


FIGURE 4 Effects of altitude (low: ■, mid: ▲, high: ●) and microsite (rock [R], tussock [T], pasture [P], bare soil [S]) on (a) recruitment, (b) seedling survival and (c) seedling height during the second growing season (t2). The symbols and bars represent the least square means and standard errors from the full models. Levels of significance for the interactions shown are denoted on top of each plot. For each model, we plotted the highest order significant interactions

was found only in pasture microsites, with no significant differences among the rest of microsites, whereas at mid-altitude rock, tussock and pasture microsites turned out to be equally unsuitable in comparison with bare soil microsites (Figure 4b; Figure S6g).

Seedling performance in terms of seedling height showed a general decrease with altitude and varying suitability between microsites as well (significant interaction altitude × microsite; Table 1). Microsite effects depended on altitude, being significant at the low altitudinal level where smallest seedlings were consistently found in bare soil, with no significant differences among the rest of microsites. For both mid- and high altitudes, no significant differences among microsites were found (Figure 4c; Figure S6h). Number of leaves was significantly affected by livestock (absent: 3.22 ± 0.17, present: 3.72 ± 0.15; least square mean ± *SE*) and microsite (R: 3.67 ± 0.20, T: 3.27 ± 0.20, P: 2.79 ± 0.23, S: 4.16 ± 0.19; significant main effects of livestock and microsite, Table 1). Number of leaves was larger in S microsites compared to P and T microsites ($S > P_{(p<0.0001)}$; $S > T_{(p=0.0009)}$). Furthermore, the proportion of stressed seedlings increased with livestock presence (absent: 48.08 ± 4.73%, present: 64.10 ± 3.59%) and differed between microsites (R: 44.44 ± 5.01%, T: 35.78 ± 5.43%, P: 68.16 ± 5.53%, S: 74.12 ± 3.52%; significant main effect of livestock and microsite; Table 1). The proportion of stressed seedlings was higher in P compared to T and higher in S compared to R and T microsites ($P > T_{(p=0.0006)}$; $S > R_{(p<0.0001)}$; $S > T_{(p<0.0001)}$).

4 | DISCUSSION

Our experiment provided important insights into the complex processes determining early life stage performance of a high mountain tree. We showed that microsite effects (at plant relevant scales sensu Purves & Law, 2002) play a fundamental role in determining tree early life performance; those effects are predominantly (but not exclusively) modulated by altitude-related changes in climate, thus varying at larger spatial scales.

4.1 | Effects of abiotic stress at altitudinal margins and livestock presence: more than their sum?

We expected that synergistic negative effects of livestock presence and altitudinal margins, commonly associated with harsher abiotic conditions, on seedling performance would be evident due to trade-offs among plant physiological responses to different environmental stressors (Archer & Stokes, 2000; Bundy, Davey, & Viant, 2009; Rhodes, Larsen, & St. Clair, 2018). Nevertheless, we did not find this particular trend in our study. No two-way interactions between altitude and livestock were detected in any of the responses during both growing seasons, indicating that the effects of livestock were independent of altitude.

Very few studies have explicitly assessed livestock presence complex interactions with altitude on tree early life performance with findings being rather divergent. Cierjacks, Rühr, Wesche and Hensen (2008), for example, reported that livestock presence significantly increased seedling density in *P. incana* at low- and mid-altitude forest stands. Marcora, Renison, País-Bosch, Cabido, and Tecco (2013) detected a stronger negative effect of livestock presence on seedling survival and growth of native woody species at low and mid-altitudes, yet, it could not be inferred whether their findings resulted from true interactive effects of altitude and livestock or from higher stocking rates at low and mid-altitudes.

Our results are in contrast to those findings. In our experimental approach, we used rather similar livestock densities along the altitudinal gradient and revealed that the impact of livestock grazing on seedling recruitment of *P. australis* is indeed independent of altitude. Although our results are confined to seedling recruitment and cannot be extrapolated to later life-cycle stages, they cover a life cycle transition of particular importance for the regeneration of mountain trees (Khurana & Singh, 2001; Schupp, 1995). Our results did also reveal that the effect of livestock presence on early recruitment of *P. australis* is much less important than the impact of microsites in interaction with altitude (see below).

4.2 | Abiotic stress and microsites: facilitative versus negative effects on early life performance

We expected that microsite effects would be mediated by altitude, with rocks or tussocks providing protection from freezing (high altitude) or desiccation (low altitude) and thus leading to higher early life performance than pasture and bare soil microsites at the altitudinal margins. This hypothesis was partially supported by our findings because in most cases, rock and tussock were most suitable at altitudinal margins. Nevertheless, in other cases (depending on estimators/growing season), pastures and soils resulted equally or more suitable for early life performance.

Differences in seedling emergence among microsites were only detected at low and high altitude. For emergence, microsite effects resulted important at both altitudinal margins where severity of conditions might to some extent be softened within the vicinity of particular microsites. At high altitude, rocks, tussocks and pastures equally favoured seedling emergence in comparison with bare soil. Many studies have also suggested that protection from strong winds and thermal buffering are likely the main mechanisms underlying the facilitative effects in high-altitude environments (Arroyo, Cavieres, Peñaloza, & Arroyo-Kalin, 2003; Cavieres, Arroyo, Peñaloza, Molina-Montenegro, & Torres, 2002; Choler, Michalet, & Callaway, 2001). On the other hand, tussocks mostly favoured seedling emergence at low altitude, and the main mechanism suggested for seedling facilitation under tussocks is the improvement of soil moisture and microclimate by the shading provided by their canopy, rather than by direct water inputs coming from run-off and the improvement of soil fertility (Maestre, Bautista, & Cortina, 2003). Under moderate conditions prevailing at mid-altitude, microsite-mediated effects shifted to net neutral, with similar suitability of all microsite types for emergence.

Later at the seedling stage, the way in which altitude influenced microsite suitability changed, as differences between microsites

were consistently insignificant at high altitude for seedling performance (t1) and recruitment. Moreover, the relative suitability of microsite types at each altitude differed depending on what response variable was considered. At low altitude, any accompanying physical structure or vegetation (i.e. rocks, tussocks, pastures), benefited both seedling height and mean number of leaves compared to bare soil conditions. Although seedlings are released from competition by existing vegetation on patches of bare soil, they obviously show signs of higher desiccation stress compared to the more sheltered microsites (pers. obs. Y. Cáceres, Table S2, Figure S3). However, regarding total proportions of recruited and surviving seedlings at low altitude, we found lowest values in pastures indicating pervasive negative effects of competition by neighbouring low-growing grasses. Grass cover might additionally intensify predation or disease of tree seedlings (Maher & Germino, 2006), but we observed little evidence for these agents. In contrast, at mid-altitude where abiotic stress factors are less limiting to growth, relative suitably of bare soil was notably increased. This tendency is particularly relevant in relation to recruitment and survival because it suggests that competition may limit seedling performance (after emergence) of *P. australis* at its optimal altitudinal distribution point, as indicated by the positive effect of neighbour removal. Other recent works based on similar experimental approaches (i.e. Frei et al., 2018; Tingstad et al., 2015) also reported positive effects of release from competition on tree seedling emergence and recruitment in bare soil microsites, across temperate treeline ecotones. Finally, at high altitude, post-emergence performance showed to be in general severely limited, with little or no differences regarding microsite type. We found that most performance responses in the first year (t1) were lowest at high altitude, including emergence, seedling height and number of leaves. In addition, seedling mortality by the second year (t2) was extremely high at highest altitude. Decreasing facilitative effects at the extreme ends of environmental gradients have been found and discussed by other authors (Kitzberger, Steinaker, & Veblen, 2010; Koyama & Tsuyuzaki, 2013; Michalet, Le Bagousse-Pinguet, Maalouf, & Lortie, 2014). The lack of microsite-mediated effects we observed on post-emergence performance (seedling performance [t1] and recruitment [t2]) at high altitude might be explained by a decline in both facilitative effects (i.e. severe environmental conditions cannot be ameliorated by surrounding vegetation or microtopographic structures) and competitive interactions (i.e. plants grow slower and smaller under extreme stress) within microsites (Le Roux & McGeoch, 2010). However, we remain cautious with this explanation given the lack of data in some of our models to infer about early life performance at high altitude.

In a wider context, our findings for the mutually dependent effects of microsite and altitude are in line with those of other authors (Klanderud, Meineri, Töpper, Michel, & Vandvik, 2017; Loranger, Zotz, & Bader, 2016; Pansing, Tomback, Wunder, French, & Wagner, 2017; Tingstad et al., 2015) highlighting that processes at the spatial scale of microsites are highly contingent on the type of abiotic stressors present in a particular environment as well as on species and the life cycle stage under consideration.

4.3 | Altitude, land use for livestock and microsite: a three-faceted influence?

The specific expectations we had regarding the presence of livestock at altitudinal margins commonly associated with harsher abiotic; exacerbating the negative effects of less suitable microsite types (pasture and soil) on early life performance was not supported. However, we found evidence that livestock-related disturbance has the potential to alter relative microsite suitability in a different way depending on altitude, having consequences on the frequency of stressed individuals (t1).

Under the drier and warmer conditions prevailing at low altitude, livestock may increase constraints on seedling recruitment (e.g. indirectly by increasing exposure of seedlings, directly by trampling), which may account for the accentuated differences in microsite suitability at low altitude. In contrast, livestock may alleviate constraints on seedling recruitment under moderate conditions at mid-altitude (e.g. by preventing light competition by tall, dominant species), which in turn may reduce differences between microsite suitability as we observed. At the colder and wetter conditions at high altitude, microsite differences were not detected regardless of livestock presence. Furthermore, the effect of livestock on height growth of seedlings depended on microsite at least in the first year: in the presence of livestock, seedling height was reduced near tussocks and in pastures, suggesting a change in growth allocation when neighbouring vegetation was grazed by livestock. These results illustrate how abiotic stress (altitudinal conditions) and biotic stress (indirectly incurred by livestock) can interact in complex ways to shift the relative importance of competition versus facilitation at the microsite level.

Although both detrimental and beneficial effects mediated by grazing have been documented and are suggested to be important for the net outcome of local plant interactions (Baraza, Zamora, & A. Hódar, 2006; Graff, Aguiar, & Chaneton, 2007; Smit, Vandenberghe, Ouden, & Müller-Schärer, 2007), future research is encouraged to study their co-occurrence along environmental gradients and to reveal the complex array of associated mechanisms underlying successful tree seedling establishment.

4.4 | Management implications

The fact that the effects of livestock grazing, microsite and altitude on emergence differed from their effects on post-emergence seedling performance remarks that shifts between facilitative and negative interactions may abruptly change across early life stage: a favourable location for a seed may quickly become an adverse one for a seedling (De La Cruz, Romao, Escudero, & Maestre, 2008). Given their widespread occurrence, seed–seedling conflicts (summarized by Schupp, 1995) likely play an important role in structuring mountain forest systems through their impacts on recruitment success. Our contribution lies in demonstrating that

changes in the relative suitability of microsites across early life stages are dependent on the interplay with altitude as well as land use for livestock. We therefore conclude that our experiment has practical implications for the restoration of mountain forests. Our results draw awareness on careful identification of specific microhabitats as "safe sites" for tree regeneration, at least in high mountain environments characterized by a remarkable spatio-temporal heterogeneity of environmental conditions. In particular, we suggest caution when interpreting results based only on a single early life stage: misleading predictions can arise if patch suitability for seeds (emergence) and seedlings (recruitment and survival) is inconsistent.

4.5 | Synthesis

In spite of the limited experimental period, our results provide important insights into how changes in environmental constrains and anthropogenic disturbance can modulate facilitative versus negative microsite effects on the early life stage performance of a mountain tree, which represent an essential component of high mountain vegetation in South America. For *Polylepis australis*, predicted climate change and intensification of land use for livestock will certainly have indirect effects on early life performance by altering local interactions at the existing microsites; general predictions regarding its future altitudinal distribution shifts are difficult to make though. In mountain gradients, changes in intensity and combination of abiotic stress type (resource based vs. non-resource based) and changes in intensity and specificities of biotic stress (foraging behaviour), microsite availability and life stage under consideration are key aspects to consider for properly assessing mountain tree regeneration success. We highlight the necessity of ample experimental effort in order to disentangle this complexity and emphasize its relevance for both ecological theory and management of mountain biodiversity.

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DATA ACCESSIBILITY

The data that support the findings of this study are openly available in "DRYAD" (www.datadryad.org); under the following digital object identifier (DOI): 10.5061/dryad.7c2g5rn. The direct link to access the data package is https://doi.org/10.5061/dryad.7c2g5rn.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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