Biotic interactions and seed deposition rather than abiotic factors determine recruitment at elevational range limits of an alpine tree

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Running headline: Biotic factors and dispersal affect tree recruitment
Summary

1. Abiotic factors, biotic interactions and dispersal ability determine the spatial distribution of species. Theory predicts that abiotic constraints set range limits under harsh climatic conditions and biotic interactions set range limits under benign climatic conditions, whereas dispersal ability should limit both ends of the distribution. However, empirical studies exploring how these three components jointly affect species across environmental gradients are scarce.

2. Here we present a study that jointly examines these factors to investigate the constraints of the recruitment of Swiss stone pine (*Pinus cembra*) at and beyond its upper and lower elevational range limits in the Swiss Alps. We investigated the natural recruitment of pines and additionally conducted seed transplant experiments to test how much abiotic factors (mean summer and winter temperatures, soil moisture), biotic interactions (understorey vegetation cover, canopy cover, seed predation) and/or seed deposition by the spotted nutcracker (*Nucifraga caryocatactes*) affect pine establishment.

3. We found significant effects of biotic interactions and seed deposition by spotted nutcrackers on the recruitment of Swiss stone pine at both the upper and lower elevational range, but could not detect significant effects of abiotic factors. Importantly, dispersal limitation rather than temperature and soil moisture restricted the recruitment of pines at the upper elevational range.

4. Synthesis. Our study highlights the importance of biotic interactions and dispersal ability in setting the upper range limits of species that have been regarded as mainly controlled by climate. This suggests that potential range shifts of plants in response to
climate warming may strongly depend on seed dispersal and biotic interactions and not only on climatic factors.

Keywords: elevational gradient, *Nucifraga caryocatactes*, *Pinus cembra*, range shifts, seed dispersal, transplant experiments
Introduction

Three important components have been distinguished to determine the geographic distribution of species: local abiotic factors, biotic interactions and the ability of species to disperse (e.g., Soberón 2007; Gaston 2009; Holt 2009). Theories that account for environmental heterogeneity suggest that abiotic constraints primarily determine range limits under harsh conditions (e.g., at high latitudes or elevations), and biotic interactions mainly determine range limits under benign conditions (e.g., at low latitudes or elevations; reviewed in Louthan et al. 2015), whereas dispersal limitation affects both ends of species’ distributions (Gaston 2009, Fig. 1). However, empirical evidence to support these theories and explore how abiotic factors, biotic interactions and species’ dispersal ability jointly affect species across large environmental gradients is scarce. In particular, quantification of the effects of biotic interactions and dispersal ability on distributions is challenging and often limited by methodological constraints (Guisan & Zimmermann 2000; Van der Putten, Macel & Visser 2010; HilleRisLambers et al. 2013).

Human-induced climate change globally modifies species’ occurrences and is predicted to intensify in the near future (Thomas et al. 2004; Pimm 2009). Already many species have responded to climate warming by shifting their ranges towards higher latitudes and elevations (Lenoir et al. 2008; Harsch et al. 2009; Chen et al. 2011; Zhu, Woodall & Clark 2012). The degree of shifts in distribution is, however, very variable among species. Until now, it is not clear to which extent climatic constraints, biotic interactions or dispersal ability explain why some species adjust to climate change by shifting their ranges and others do not (Thuiller 2004; Angert et al. 2011). Few studies go beyond the comparison between current and previous range limits and empirically test the
mechanisms of range shifts. Several studies suggest that non-climatic factors, such as biotic interactions or dispersal ability, could explain the variable responses of species to changing climates (Zhu et al. 2012; HilleRisLambers et al. 2013). Modelling approaches have attempted to incorporate biotic interactions (Guisan & Thuiller 2005; Meier et al. 2010; Puerta-Piñero, Pino & Gómez 2012) and dispersal ability (Westcott et al. 2005; Russo, Portnoy & Augspurger 2006; Uriarte et al. 2011) in models of range dynamics and have demonstrated their potential importance in shaping species’ distributions. Transplant experiments, especially of plants, comparing the fitness and survival ability of the species within and beyond their ranges, have further been used to infer potential dispersal limitation of species under the effects of changing climates (Hargreaves, Samis & Eckert 2014; Dvorský et al. 2016). To better understand the mechanisms of range dynamics in response to environmental change, simultaneous empirical testing of all three components is necessary.

We present a study jointly investigating all three components. We examined how abiotic factors, biotic interactions and dispersal ability determine the establishment of the Swiss stone pine (*Pinus cembra* L.), a keystone tree species of subalpine forests of the treeline ecotone. We investigated the establishment of Swiss stone pine along its elevational distribution, because elevational gradients allow the study of broad environmental conditions on rather small geographic scales (Popy, Bordignon & Prodon 2010). Swiss stone pine has its largest distribution in the central European Alps, where it co-occurs with European larch (*Larix decidua* Mill.) and often forms the treeline. It occurs across a short elevational gradient ranging from about 1500 – 2400 m a.s.l. (Ulber, Gugerli & Bozic 2004). At its lower elevational range limit, it is outcompeted by other
tree species, in particular spruce *Picea abies* (L.) H. Karst.). The pine lives in close interaction with its primary disperser, the spotted nutcracker *Nucifraga caryocatactes* (Linnaeus, 1758)], which is the only animal that may open the closed cones of the pine and extract the seeds to store them in caches beneath the soil surface for later consumption (Neuschulz et al. 2015). Hence, these birds are the only significant dispersal vectors of Swiss stone pine. The global distribution of spotted nutcrackers is larger than that of Swiss stone pine (Mattes et al. 1982), indicating that pine occurrence beyond its distribution edges might not necessarily be restricted by the presence of its disperser. In fact, distribution models of the tree species have suggested that Swiss stone pine is strongly influenced by climatic factors rather than by biotic constraints (Meier et al. 2010).

In this study, we combined field observations of the natural recruitment of pines and seed transplant experiments to investigate how abiotic factors (i.e., mean summer and winter temperatures, soil moisture), biotic interactions (i.e., understorey vegetation cover, canopy cover, rodent seed predation) and dispersal ability (i.e., seed deposition by spotted nutcrackers) constrain the recruitment of Swiss stone pine. We show bird-mediated seed deposition, which in the strict sense is a biotic interaction, separately from other biotic interactions to better illustrate the three components determining species occurrence. We tested how the three components affect pine recruitment and establishment across and beyond its entire elevational range and separately at its lower and upper range limits. The deposition of seeds by nutcrackers and the absence of other effective dispersal vectors, such as wind, offers the unique opportunity to study the dispersal ability of an animal-dispersed tree. We hypothesised that abiotic factors, such as temperature, would control
the occurrence of the pine at the upper elevational range where climatic conditions are
harsh (Vittoz et al. 2008; Meier et al. 2010; Boden, Pyttel & Eastaugh 2010), whereas
biotic controls, such as plant–plant interactions would affect the occurrence of the pine at
its lower elevational range where climatic conditions are benign (Ulber et al. 2004). We
expected little dispersal limitation at both ends of the pine’s range, due to the efficiency
and mobility of its seed disperser, the spotted nutcracker (Mattes 1982).

Materials and methods

Study area and design

The study was conducted within the core distribution of Swiss stone pine in the eastern
Swiss Alps, in the Canton of Grisons (Fig. S1). Field sites were located in two valleys
close to Davos, Flüela (46°48’0.25''N, 9°54’15.38''E) and Sertig (46°44’0.76''N,
9°51’3.50''E). In both valleys, Swiss stone pine forms the upper treeline and occurs at
elevations from about 1850 m to 2200 m a.s.l. Highest pine densities occur at
intermediate elevations within the elevational range. The treeline (upper elevational limit
at which trees reach three metres in height, Harsch et al. 2009) reaches 2150 m a.s.l., but
“krummholz” trees are found up to 2200 m a.s.l. The lower elevational range of Swiss
stone pine is dominated by spruce (Picea abies) forest.

Abiotic factors, biotic interactions and seed deposition

We tested the effect of 1) abiotic factors (i.e., mean summer and winter temperatures, soil
moisture), 2) biotic plant–plant and plant–animal interactions (i.e., understorey vegetation
cover, canopy cover, seed predation) and 3) dispersal ability (i.e., seed deposition by
spotted nutcrackers) that in previous publications have been studied in isolation and were
considered to be the most important factors for the regeneration of Swiss stone pine (e.g.,
Vittoz et al. 2008; Zurbriggen et al. 2013). We focused on the pine’s early life stages, as
the ability of a plant to establish under new environmental conditions is key to respond to
potential climatic changes (Vitasse et al. 2012). 1) Abiotic factors: Low summer and
winter temperatures are restrictive for plant establishment and growth at the treeline,
restricting tissue formation of plants in the growing season (e.g., Rossi et al. 2007) and
cause... (Vittoz et al. 2008). Soil water content is an indicator for potential drought stress of plants (Anfodillo et al. 1998). Shallow and
permeable soils that frequently occur at high elevations often cause dry soil conditions
(Anfodillo et al. 1998), which can negatively affect seedling establishment (Moyes et al.
2012). 2) Biotic interactions: Understorey vegetation cover provides shelter for seedlings
and therefore is particularly important for plant establishment in harsh alpine
environments (Graae et al. 2011; Mamet & Kershaw 2013). In contrast, dense shrub
cover can also impair seedling growth, because of high interspecific competition (Liang
et al. 2016). Canopy cover determines the availability of light, which is essential for the
photosynthetic activity of plants. At the same time, elevated UV-B radiation, which is
particularly severe at sites with low canopy cover at high elevations, may negatively
affect seedling establishment processes (van de Staaij et al. 1997). Finally, the predation
of seeds, for instance by rodent seed predators, is an essential determinant of regeneration
across and beyond plant range limits that may strongly reduce seedling establishment
(e.g., Castro et al. 1999; Brown & Vellend 2014). 3) Dispersal ability: Many plants
depend on animals for the dispersal of their seeds. The dispersal and deposition of seeds
by spotted nutcrackers is of particular importance for the regeneration of Swiss stone pine, as its cones, in adaptation to bird dispersal, remain closed on the tree until harvested by the birds. Nutcrackers are the only animals that may open the cones and extract the seeds with their strong bills (Mattes 1982). Therefore, Swiss stone pine strongly depends on seed-caching nutcrackers for regeneration.

**Natural recruitment of Swiss stone pine**

We monitored the natural recruitment of Swiss stone pine seedlings and saplings across and beyond its elevational distribution in both the Flüela and Sertig valleys in September for three consecutive years, from 2012 to 2014. In each valley and year, we established 9 rectangular 20 m² plots at randomly selected locations between 1850 and 2250 m a.s.l., spaced by 50 m vertical height difference. Plots at 2250 m a.s.l. were established beyond the upper range limit of Swiss stone pine. In each year, the 20-m² plots were placed at different locations, resulting in 54 20-m² plots and a total area of 1080 m² sampled over the three years. We recorded the total number of seedlings and saplings up to 1 m height per plot. Due to the variable growth form of pine juveniles, it was not possible to classify different age classes of seedlings and saplings in a non-destructive way.

To record the abiotic conditions, biotic interactions and seed deposition for each 20 m² plot, we divided each plot into 20 1-m² sub-plots. To obtain mean summer and winter temperatures, we classified each sub-plot into one of the following four microhabitat types: open soil, dwarf vegetation, rocky habitat, and close to tree trunk. We measured temperature with 531 data loggers (Maxim iButton) that recorded soil surface temperatures every four hours over the entire 3-year study period. Temperature data
loggers were equally distributed across all nine elevational bands (i.e., 1850 – 2250 m) in both valleys, covering the four microhabitat types at each elevation. We calculated the mean, minimum and maximum temperature of the warmest three months (June–August) and the mean, minimum and maximum temperature of the coldest three months (December–February) for each microhabitat type at each elevational band per valley over the study period. We estimated the temperature for each of the 20 sub-plots based on its microhabitat type, elevation, valley and year, and calculated average summer and winter temperatures over all 20 sub-plots for each 20 m² plot. As minimum and maximum temperatures were correlated to the mean of summer and winter temperatures, we did not use these variables in the analyses. We also calculated growing degree-days (GDD) according to the formula GDD = [(Tmax + Tmin) / 2] – Tbase, where Tmax / Tmin are daily maximum and minimum temperatures and Tbase is the temperature below which plant growth does not progress (McMaster & Wilhelm 1997). We set Tbase to 5 °C according to Körner & Paulsen (2004). Any daily minimum temperature below Tbase was set to Tbase before calculating the average. Mean soil moisture per plot was recorded by averaging measurements of soil moisture (in percent) over all 20 sub-plots. Soil moisture measurements were taken each year in September under dry weather conditions by averaging five tensiometer (Theta-Kit version 3) measurements, sampled within 5 cm of the centre of each sub-plot.

Mean understorey vegetation cover per plot was recorded by averaging measurements of dwarf shrub vegetation, such as European blueberry (*Vaccinium myrtillus* L.), common juniper (*Juniperus communis* L.) and alpenrose (*Rhododendron ferrugineum* L.), within each sub-plot according to Braun-Blanquet (1964). Mean canopy
cover per plot was recorded by averaging the canopy cover of each of the 20 sub-plots sampled by a spherical crown densitometer (Forestry suppliers).

To obtain a comparable estimate of the number of seed caches deposited by nutcrackers per 20 m² plot, we took 1-dm³ soil samples in the centre of each of the 20 sub-plots in which we thoroughly searched for seed caches established by the birds (i.e., 1080 soil samples total over the three years, Briggs, Vander Wall & Jenkins 2009). A previous study has shown that this method reveals the same patterns of seed deposition as recorded by focal observations of seed-caching nutcrackers (Neuschulz et al. 2015). If an intact seed or a seed shell handled by a nutcracker was present in the soil sample, we recorded cache presence and later summed the overall number of caches per plot. As nutcrackers are the main dispersal vector for Swiss stone pine seeds, the presence of seeds in the soil is a clear indication for seed deposition by nutcrackers.

Experimental seedling establishment

To investigate the potential of pine seedling establishment across and beyond the current elevational range of the pine, we conducted seed transplant experiments in the years 2012 and 2014. We deployed 90 experimental caches in each of the two valleys in May after snowmelt, resulting in a total number of 360 experimental caches in both valleys over the study period of two years. We chose the time after snowmelt, as this is the typical time when Swiss stone pine seedlings start to germinate under natural conditions. At each of the nine elevations, ranging from 1850 to 2250 m a.s.l., and spaced again by 50 m vertical height difference, we deployed caches in five microhabitat types (open soil; dwarf vegetation; rocky habitat; close to tree trunks; microsite covered by snow).
Experimental caches were placed close to, but not at the same sites as the 20 m² plots of natural pine recruitment. Due to the absence of trees at high elevation plots (2250 m a.s.l.), we exchanged the microhabitat “close to tree trunk” by sites in dense matgrass cover (*Nardus stricta*). We intentionally followed this random-stratified design to comprehensively cover the environmental space available at the selected mountain slopes. Two replicates per microhabitat were established, resulting in 10 experimental caches at each of the 9 elevations per year. Each cache contained five seeds (total number of seeds over two years = 1800), placed 4 cm deep in the soil in a soil-filled mesh bag that was open at the top and fixed by metal pins. We chose the number of five seeds as this was the average number of seeds recorded for seed caches established by spotted nutcrackers (*Mattes* 1982). Prior to the experiments, seeds had been treated to break dormancy (i.e. stored in a moistened 1:1 clay–sand mixture under varying temperatures for 10 weeks), which would naturally happen during winter time. Three months after planting, we checked whether seedlings had established (germinated or established seedlings), seeds had been predated or removed by rodents (gnawed or missing seeds), or seeds remained intact.

At every experimental cache, we measured the mean summer temperature, GDD, mean soil moisture, canopy cover and understorey vegetation cover (methods described in the section above). Mean temperature of the warmest three months of the growing season (June–August) was recorded using 270 data loggers that were installed at experimental caching sites and recorded soil surface temperatures every four hours. In 2012, data loggers were installed at one out of two experimental caches per microhabitat at each elevation and valley, whereas in 2014 data loggers were installed at each
experimental cache. We also measured the proportions of ground-layer cover of grass, pine needles, bare ground and nutrient availability at each experimental cache to capture the fine-scale seedbed conditions. As grass and needle cover were significantly negatively correlated to understorey dwarf shrub vegetation cover (grass cover: Pearson’s r = -0.68, t = -6.76, d.f. = 52, p < 0.001; needle cover: Pearson’s r = -0.50, t = -3.83, d.f. = 52, p < 0.001), and bare ground and nutrient availability were significantly correlated to soil moisture (bare ground: Pearson’s r = -0.34, t = -2.63, d.f. = 52, p = 0.011; nutrient availability: Pearson’s r = 0.60, t = 4.36, d.f. = 52, p < 0.001), we did not use these variables in the analyses.

Data analyses
First, we modelled natural pine recruitment (i.e., number of juvenile plants per plot) and experimental seedling establishment (i.e., probability of seedlings to establish from all sown seeds) as a function of elevation using generalized linear models (GLMs). We modelled natural pine recruitment assuming a quasi-Poisson error distribution accounting for overdispersion and experimental seedling establishment assuming a binomial error distribution. Both models included a quadratic and linear term of elevation as fixed effects and the valley and the study year as fixed effect control variables. We selected the best model based on Akaike’s information criterion for small sample size (AICc, Hurvich and Tsai 1989) and its equivalent for overdispersed models (qAICc, Bartón 2015). In the model of natural pine recruitment, we retained a linear and a quadratic term of elevation and in the model of experimental seedling establishment, we retained a linear term of elevation.
Second, we modelled each abiotic factor, biotic interaction and seed deposition measured in the recordings of natural and experimental pine recruitment as a function of elevation using GLMs. All models also included the valley and the study year as fixed effect control variables. Mean summer and winter temperatures were modelled assuming a normal distribution. Soil moisture, understorey vegetation cover, canopy cover, and seed predation (i.e., proportion of predated seeds per cache) were modelled assuming a quasi-binomial error distribution accounting for overdispersion. Seed deposition (i.e., number of seed caches) was modelled assuming a quasi-Poisson error distribution accounting for overdispersion. In all models, we fitted a quadratic and linear term of elevation and selected the best model based on Akaike’s information criterion for small sample size (AICc, Hurvich and Tsai 1989) and its equivalent for overdispersed models (qAICc, Bartón 2015). We retained both the quadratic and linear term of elevation in all models, except in the models of mean summer temperature and seed predation in which only the linear term of elevation was retained.

Third, we tested the importance of all abiotic factors, biotic interactions and seed deposition as predictors for natural pine recruitment and experimental seedling establishment across and beyond the entire elevational range of Swiss stone pine (1850 m – 2250 m a.s.l.). To quantify and compare the predictor’s importance at the lower and upper range limit of the species, we also separately analysed the lower range (< 2050 m a.s.l., four elevational bands) and upper range (> 2050 m a.s.l., four elevational bands). Prior to these analyses, we tested for collinearity of the predictor variables. As understorey vegetation cover was significantly negatively correlated with canopy cover (natural pine recruitment: Pearson’s r = -0.50, t = -4.06, d.f. = 49, p < 0.001; transplant
experiments: Pearson’s $r = -0.46$, $t = -9.69$, d.f. = 343, $p < 0.001$), we excluded canopy cover from the final analyses. We also excluded GDD from the final analyses due to significant positive correlation to mean summer temperature (natural pine recruitment: Pearson’s $r = 0.71$, $t = 7.20$, d.f. = 52, $p < 0.001$). However, we additionally present models including canopy cover (instead of understorey vegetation cover) and GDD (instead of mean summer and winter temperatures) in the Appendix. To provide comparability of the predictor estimates among each other, all predictor variables were centred and standardized using Gelman’s approach prior to the analysis using the arm package (Grueber et al. 2011; Gelman 2016). We fitted full GLMs including all combinations of the predictor variables of the models using the MuMIn package (Bartón 2015). Models of natural pine recruitment included mean summer temperature, mean winter temperature and soil moisture, understorey vegetation cover (canopy cover, respectively), and the number of caches and were modelled assuming a quasi-Poisson error distribution accounting for overdispersion. Models of experimental seedling establishment included mean summer temperature, soil moisture, seed predation, and understorey vegetation cover (canopy cover, respectively) and were modelled using a quasi-binomial error distribution accounting for overdispersion. All models also included the valley and the study year as fixed effect control variables. First, we obtained the relative importance of each predictor variable in the full models. We conducted model averaging by using the standardized regression coefficients of all candidate models weighted by the model’s qAICc weights. The variable importance values of all predictor variables were then scaled to sum up to one for comparison between models of natural pine recruitment and experimental seedling establishment. Second, we selected the best
models based on $\Delta q\text{AIC}_c < 5$ and calculated model estimates based on model averaging of these best models (Bartón 2015).

Results

Natural and experimental recruitment of Swiss stone pine across the elevational gradient

We recorded 135 pine seedlings and saplings in 54 20-m$^2$ plots across the elevational gradient in both valleys and all years. The natural recruitment of pines showed a hump-shaped distribution with the highest predicted number of four juveniles per 20-m$^2$ plot at 2070 m a.s.l. averaged across both valleys (Fig. 2a).

In the transplant experiments, 193 seedlings established out of 1800 seeds planted over two years. Unlike the natural recruitment of pines, seedling establishment in the transplant experiments increased with increasing elevation (Fig. 2b). While the predicted probability of seedling establishment was only 3% at the lower edge of the elevational range at 1850 m a.s.l., the probability of seedling establishment was 17% beyond the current distribution of the pine at 2250 m a.s.l.

Abiotic factors, biotic interactions and seed deposition across the elevational gradient

Patterns of abiotic factors, biotic interactions, and seed deposition measured at both observational plots and experimental sites significantly differed across the elevational gradient (Figs 3 and S2, Tables S1 and S2). Mean summer temperature significantly increased with increasing elevation, reflecting the high solar radiation that seedlings experience in summer at high elevations, which was measured by the temperature loggers at the soil surface (Figs 3a and S2a, Tables S1 and S2). Mean winter temperature showed
a slight, but non-significant decrease across the elevational gradient (Fig. 3b, Tables S1).

Soil moisture showed a u-shaped distribution across the elevational gradient, with lowest values at mid-elevations (Figs 3c and S2b, Tables S1 and S2). While canopy cover significantly decreased across the elevational gradient (Fig. S2c, Tables S1 and S2), understorey vegetation cover significantly increased with elevation (Figs 3d and S2d, Tables S1 and S2). Out of 1800 seeds planted in the transplant experiments over two years, 764 seeds were preyed upon or removed by rodents. Seed predation was high at low elevations (predicted probability of 74 % at 1850 m a.s.l.) and significantly declined across the elevational gradient (predicted probability of 28 % at 2250 m a.s.l., Fig. 3e, Table S2). In 152 out of 1080 soil samples, we recorded seed caches deposited by spotted nutcrackers. Seed deposition followed a hump-shaped distribution, with the highest predicted number of 4.9 caches per plot at 2025 m a.s.l. averaged across all valleys and years (Fig. 3f, Tables S1).

Relative importance of abiotic factors, biotic interactions, and seed deposition for natural and experimental pine recruitment

Seed deposition and biotic interactions explained more than half of the relative importance of factors affecting the natural recruitment of pines and experimental seedling establishment across and beyond the elevational range of the tree (Fig. 4). Abiotic factors contributed with a relative importance of 0.23 in explaining the natural recruitment of pines across elevations. In contrast, understorey vegetation cover and seed deposition together had a relative importance of 0.72 (Fig. 4a). A similar pattern was found in the transplant experiments where abiotic factors contributed with a relative importance of
0.13 in explaining experimental seedling establishment. In contrast, understorey vegetation cover and seed predation together had a relative importance of 0.86 (Fig. 4b). At the upper range of the distribution of Swiss stone pine, the relative importance of abiotic factors was 0.16 in explaining natural pine recruitment and 0.10 in explaining experimental seedling establishment, whereas the relative importance of seed deposition and biotic interactions was 0.67 for natural pine recruitment and 0.83 for experimental seedling establishment (Fig. 4a, b “Upper range”). At the lower range of the distribution of Swiss stone pine, the relative importance of abiotic factors was 0.42 in explaining natural pine recruitment and 0.07 in explaining experimental seedling establishment, whereas the relative importance of seed deposition and biotic interactions was 0.06 for natural pine recruitment and 0.89 for experimental seedling establishment (Fig. 4a, b “Lower range”). All models using canopy cover instead of understorey vegetation cover showed qualitatively similar results (Fig. S3). This pattern was confirmed by qAIC-based model selection, showing significant effects for understorey vegetation cover, canopy cover, seed predation and seed deposition on natural pine recruitment and experimental seedling establishment (Table 1, Table S3). Abiotic factors were not significant in all the best models of Swiss stone pine recruitment (Table 1, Table S3).

**Discussion**

To date, there is little empirical evidence of how abiotic factors, biotic interactions, and dispersal ability jointly determine range limits of plants (but see Puerta-Piñero et al. 2012; Brown & Vellend 2014). Many previous studies have attributed range limits especially in harsh environments to abiotic conditions (e.g., Normand et al. 2009; Lee-
Yaw et al. 2016) and projections of potential range shifts under climate change are often solely based on abiotic factors (e.g., Shevtsova et al. 2009; Engler et al. 2011). We here provide a comprehensive study on species range limits that includes all three components. We find evidence that seed deposition and biotic interactions determined the recruitment of Swiss stone pine and not, as expected, harsh abiotic conditions at the upper range limit (Vittoz et al. 2008; Boden et al. 2010). Transplant experiments demonstrated that seedlings could establish above the current tree line. However, patterns of seed deposition by nutcrackers suggested that dispersal to high elevations might be rare and seeds do not frequently reach locations beyond the treeline. Our findings demonstrate that understanding the underlying mechanisms of dispersal ability and biotic interactions is, beside the consideration of abiotic factors, important for projecting potential range shifts of plants under climate change.

Spotted nutcrackers cached seeds primarily within the range limits of the current elevational distribution of the pine, which appeared to be the main factor restricting the elevational range of the tree species. These findings were supported by the high probability of pine seedling establishment beyond the treeline in the transplant experiments, indicating potential dispersal limitation of the tree. Our results were surprising, as plants growing at steep elevational gradients are generally not regarded as dispersal limited because of the small spatial extent of a large variety of different habitats (Jump, Mátyás & Peñuelas 2009; Hargreaves et al. 2014; Siefert, Lesser & Fridley 2015).

Spotted nutcrackers are able to conduct far-ranging seed dispersal flights among valleys (Mattes 1982) and their high mobility should not restrict pine seed dispersal. Several reasons could explain the low seed deposition beyond the current limits of pine
distribution: Spotted nutcrackers prefer microsites for seed caching where the perishability of stored seeds is low, hence the probability of seedling establishment is low (Neuschulz et al. 2015). Nutcrackers thus might avoid caching seeds beyond the treeline as these seeds have a high probability of establishment, which would spoil them as a potential food source. Also, the lack of landmarks such as trees beyond the treeline (Smulders, Gould & Leaver 2010) and the very deep snow at high elevation sites during winter (Mattes 1982) could hinder the recovery of seed caches by the birds. Despite the low seed deposition at high elevations, juvenile pines occurred in our study area at elevations up to 2200 m a.s.l. An explanation for this high elevation occurrence could be the synchronous production of above-average seed-crops every few years, which is typical for Swiss stone pine (Zong et al. 2010), but which we did not encounter in our study period. Animals have been shown to disperse seeds in mast years more frequently and farther compared to typical seed-production years (Vander Wall 2002). While the probability of seed deposition beyond the treeline averaged over several years might be low, nutcrackers might expand their caching sites to high elevations in mast seeding years. In summary, our results underpin that potential upwards movements of the Swiss stone pine under climate warming may strongly depend on the caching behaviour of its main disperser, the spotted nutcracker. While the close mutualistic interaction between nutcracker and pine gave us the unique opportunity to study the dispersal ability of the tree species in great detail, we are aware that the prominent role of seed deposition by spotted nutcrackers for pine establishment might be specific for this highly specialized plant–bird system. However, previous studies that have investigated the often neglected spatially-explicit patterns of seed deposition across heterogeneous environments show the
effects of distinct and non-random deposition of seeds by different dispersal vectors, such as wind or animals (e.g., Gómez-Aparicio, Gomez & Zamora 2007; Damschen et al. 2014; Mueller et al. 2014). We therefore believe that seed deposition might be more important for local range expansions than previously assumed.

Interestingly, not only seed deposition, but also biotic interactions (i.e., understorey vegetation and the lack of seed predation) influenced Swiss stone pine establishment at the upper range of the species more than abiotic factors, which contrasts the long-standing prediction that climate sets the range limits in abiotically stressful environments (e.g., reviewed in Louthan et al. 2015, Fig. 1). Recent studies provide increasing evidence that biotic interactions are often equally important as abiotic factors for plant establishment at high elevations (HilleRisLambers et al. 2013; Brown & Vellend 2014; Tingstad et al. 2015; Liang et al. 2016). Dense shrub cover has, for instance, been shown to reduce seedling establishment at treeline ecotones (Tingstad et al. 2015) and thus, may decelerate the potential upwards movements of tree species under climate change (Liang et al. 2016). In our study, understorey vegetation cover fostered the natural recruitment and experimental establishment of Swiss stone pine. Plants growing in stress-prone environments often experience ameliorated microhabitat conditions in dense shrub vegetation (e.g., Callaway et al. 2002; Gómez-Aparicio et al. 2004), such as beneficial soil conditions (HilleRisLambers et al. 2013), shelter and higher humidity (Graae et al. 2011) or reduced herbivory (Cairns & Moen 2004). Seed predation by rodents strongly reduced the establishment of pine seedlings. Similar patterns have been shown by Brown and Vellend (2014) who found that high rates of rodent predation on seeds of sugar maple (Acer saccharum) prevented the potential upward movement of
the tree species. In contrast to their findings, we found that seed predation was much higher (over 70 %) at the lower range edge of Swiss stone pine compared to that beyond the treeline (30 %), likely due to lower rodent densities at high elevations (McCain 2005). Our results emphasise that biotic interactions play a crucial role in determining the occurrence of plants across environmental gradients.

Temperature has widely been considered to control plant distribution limits (e.g., Normand et al. 2009, Vitasse et al. 2012, Louthan et al. 2015). While many studies have shown that summer temperatures determine the formation of altitudinal and latitudinal treelines (Körner & Paulsen 2004, Holtmeier & Broll 2007, Siefert et al. 2015), there is also evidence that winter temperatures are decisive for treeline formation (e.g., Rickebusch et al. 2007, Harsch et al. 2009). Although the natural climatic treeline in the Alps has been shifted downslope due to long-lasting anthropogenic disturbances (Gehrig-Fasel, Guisan & Zimmermann 2007, Holtmeier & Broll 2007), climatic controls of plant establishment and growth prevail (Holtmeier & Broll 2007, Vitasse et al. 2012). For instance, seedlings of Swiss stone pine have been reported to suffer in particular from extreme frost events (Barbeito et al. 2012) whereas long and cold winters may cause severe growth depression and damage of juvenile and adult trees (Vittoz et al. 2008). In our study, we did not find a significant effect of mean summer and winter temperatures on the natural recruitment and experimental seedling establishment of Swiss stone pine, neither across the entire elevational range nor at the upper range of the species. Although winter temperature had a rather large relative importance in some of the models explaining the natural recruitment of pines, its effects were not significant (Table S1). We might have underestimated the effect of winter temperatures in our relatively short three-
year study, because cold winter events that could potentially affect pine populations in the study area take place roughly every 4-5 years (Beniston 2012). Long-term studies of climate effects on the establishment and survival of plants should be the focus of future studies (Dvorský et al. 2016). Climate manipulation experiments in the field could further be used to simulate the long-term effects of climate warming on seedling establishment (De Boeck et al. 2015; Elmendorf et al. 2015). Yet, our study emphasises that in the short-term, seed deposition by spotted nutcrackers and biotic interactions appear to control the establishment and survival of Swiss stone pine across and beyond its upper distribution limit.

Conclusion

Explaining the distribution of plants across environmental gradients has been a great challenge in past research. Here we show that seed deposition and biotic interactions determine the recruitment of an alpine tree species, the Swiss stone pine. Our findings suggest that dispersal ability and biotic interactions may be dominant factors affecting the early stages of the plant life cycle, particularly if plants depend on animal dispersal. This has important implications for understanding species range dynamics along environmental gradients, revealing that biotic interactions and dispersal ability are, beside the already known abiotic factors, also important for explaining the occurrence of plants. Our findings may not only apply to elevational, but also to latitudinal range limits of plants. We expect that especially dispersal ability might control the occurrence of plants at their latitudinal limits, because dispersal limitation has been shown to be even more restrictive for plant range expansion at latitudinal than at elevational range edges (Siefert
et al. 2015). We believe that our findings have key implications for understanding the range dynamics of plants growing along environmental gradients, emphasising that seed deposition might be more important for local range expansions than previously assumed.

Authors Contribution

E.L.N. and K.G.B. conceived the ideas and designed methodology. E.L.N. and D.M. collected the data. E.L.N. analysed the data and led the writing of the manuscript. E.L.N., D.M., K.B., F.G. and K.G.B. contributed to the various drafts and gave final approval for publication.

Acknowledgements

We thank all landowners that allowed us to work on their property. This work would not have been possible with the enormous help of Lisa Braasch, Mathias Brummer, Simon Crameri, Maximilian Fader, Sarah Göttlich, Felix Günther, Anke Hempel, Stephan Kambach, Oliver Kruse, Annika Licht, Johanna Müller, Felix Närmann, Pablo Stelbrink, Mathias Templin and the Shima Davos. Anton Burkart from the Swiss Federal Institute for Forest, Snow and Landscape Research (WSL) provided seeds of Swiss stone pine. We thank Diana Bowler, Matthias Schleuning and two anonymous reviewers for comments on a previous version of this manuscript. FG acknowledges support from the Swiss National Science Foundation (PiCadapt, 31003A_152664). ELN acknowledges support from the Daimler and Benz Foundation and the German research foundation (DFG).

Data accessibility
Data from this paper are deposited in the Dryad Digital Repository doi:10.5061/dryad.8t2q2 (Neuschulz et al. 2017).
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recruitment of European tree species at their current upper elevational limits in the


Table 1. Effects of abiotic factors, biotic interactions and seed deposition on the number of juveniles a) and the probability of experimental seedling establishment b) across the elevational range and at the upper and lower range of Swiss stone pine. Generalized linear models were fitted assuming quasi-Poisson a) and quasi-binomial b) error distributions accounting for overdispersion. Estimates present model averages from qAICc-based model selection.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Estimate</th>
<th>SE</th>
<th>p value</th>
</tr>
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<tr>
<td>a) # juvenile plants</td>
<td>~ Seed deposition</td>
<td>0.85</td>
<td>0.27</td>
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<tr>
<td>Across range</td>
<td>+ Understorey vegetation</td>
<td>1.01</td>
<td>0.48</td>
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<tr>
<td>(1850 – 2250 m a.s.l.)</td>
<td>+ Winter temperature</td>
<td>0.38</td>
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</tr>
<tr>
<td></td>
<td>+ Summer temperature</td>
<td>0.03</td>
<td>0.21</td>
</tr>
<tr>
<td></td>
<td>+ Soil moisture</td>
<td>0.06</td>
<td>0.20</td>
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<td></td>
<td>+ Region</td>
<td>0.04</td>
<td>0.20</td>
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<tr>
<td></td>
<td>+ Year 2013</td>
<td>-0.01</td>
<td>0.10</td>
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<td>+ Year 2014</td>
<td>-0.00</td>
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<tr>
<td>Upper range</td>
<td>~ Seed deposition</td>
<td>1.95</td>
<td>0.55</td>
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<tr>
<td>(&gt; 2050 m a.s.l.)</td>
<td>+ Understorey vegetation</td>
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<tr>
<td></td>
<td>+ Winter temperature</td>
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<td>+ Soil moisture</td>
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<td></td>
<td>+ Region</td>
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<td>+ Year 2013</td>
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<td></td>
<td>+ Year 2014</td>
<td>0.52</td>
<td>0.66</td>
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<tr>
<td>Lower range</td>
<td>~ Seed deposition</td>
<td>0.11</td>
<td>0.32</td>
</tr>
<tr>
<td>(&lt; 2050 m a.s.l.)</td>
<td>+ Understorey vegetation</td>
<td>-0.08</td>
<td>0.35</td>
</tr>
</tbody>
</table>
+ Winter temperature   0.53   0.80   0.519
+ Summer temperature  -0.68   0.89   0.452
+ Soil moisture       0.55   1.02   0.596
+ Region              0.51   0.67   0.458
+ Year 2013           -0.03   0.69   0.966
+ Year 2014           -1.55   1.35   0.259

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<th></th>
<th>+ Winter temperature</th>
<th>+ Summer temperature</th>
<th>+ Soil moisture</th>
<th>+ Region</th>
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<td>+ Year 2014</td>
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<td>0.259</td>
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b) Seedling establishment ~ Seed predation -2.45 0.30 < 0.001 ***
Across range + Understorey vegetation 0.51 0.17 0.002 **
(1850 – 2250 m a.s.l.) + Summer temperature 0.16 0.19 0.429
+ Soil moisture 0.28 0.22 0.189
+ Region 0.01 0.10 0.937
+ Year 0.02 0.12 0.852

Upper range ~ Seed predation -1.73 0.37 < 0.001 ***
(> 2050 m a.s.l.) + Understorey vegetation 0.29 0.27 0.296
+ Summer temperature -0.00 0.13 0.987
+ Soil moisture 0.23 0.27 0.379
+ Region 0.05 0.15 0.752
+ Year 0.12 0.23 0.590

Lower range ~ Seed predation -2.89 0.49 < 0.001 ***
(< 2050 m a.s.l.) + Understorey vegetation 0.11 0.22 0.598
+ Summer temperature 0.18 0.26 0.478
+ Soil moisture 0.06 0.18 0.730
+ Region -0.05 0.17 0.794
+ Year -0.05 0.19 0.784
Fig. 1. Conceptual illustration of the three key determinants of species range limits: abiotic factors, biotic interactions and dispersal ability (e.g., Soberón 2007; Holt 2009). While abiotic factors are predicted to limit species distributions in particular under stressful abiotic conditions and biotic interactions should limit distributions under benign abiotic conditions (Louthan et al. 2015), dispersal should be limiting at both ends of species’ ranges (Gaston 2009).
Fig. 2. Natural recruitment of Swiss stone pine (a) and probability of seedling establishment in transplant experiments (b) as a function of elevation. In the generalized linear model of natural pine recruitment, a linear and a quadratic term were fitted assuming a quasi-Poisson error distribution accounting for overdispersion. In the generalized linear model of experimental seedling establishment, a linear term was fitted assuming a binomial error distribution. Circles indicate jittered raw data. Grey lines show model fit for the different valleys and years, the red line shows the overall model fit ($p < 0.05$). Note break in y-axis of the experimental establishment model.
Fig. 3. Abiotic factors (a-c), biotic interactions (d-e), and seed deposition (f) recorded in the sampling of natural pine recruitment (a-d, f) and seed transplant experiments (e) as functions of elevation; (a) mean soil surface temperature of the three warmest months, (b) mean soil surface temperature of the three coldest months, (c) proportion of soil moisture, (d) proportion of understorey vegetation cover, (e) seed predation by rodents, (f) seed deposition by spotted nutcrackers. Generalized linear models were fitted using a linear (a) and a linear and quadratic term of elevation (b) and assuming a Gaussian error distribution. In (e) a linear and in (c-d) a linear and a quadratic term were fitted assuming a quasi-binomial error distribution accounting for overdispersion. In (f) a linear and quadratic term was fitted assuming a quasi-Poisson error distribution accounting for overdispersion. Circles indicate jittered raw data. Grey lines show model fit for the different valleys and years, the red line shows the overall model fit (solid: p < 0.05, dashed: p > 0.05). Further abiotic factors and biotic interactions recorded in seed
transplant experiments are presented in Fig. S2. Model statistics are presented in Table S1 and S2.
Fig. 4. Relative importance of abiotic factors (violet), biotic interactions (green) and seed deposition (blue) on the natural recruitment of Swiss stone pine (a) and the probability of seedling establishment in transplant experiments (b) across the elevational range. Models included the entire elevational range (1850 – 2250 m a.s.l.) and the lower (< 2050 m a.s.l.) and upper range (> 2050 m a.s.l.) of pine distribution. +/- indicate the direction of the most important effects. Seed = seed deposition by spotted nutcrackers, Veg = understorey vegetation cover, Predation = seed predation by rodents, SummerT = mean temperature of the warmest three months, WinterT = mean temperature of the coldest three months, SoilMoist = soil moisture, Year = study year, Valley = study valley.