



Microsite preferences of three conifers in calcareous and siliceous treeline ecotones in the French alps

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Abstract

Seedling establishment is crucial for elevational advance of tree species above the treeline ecotone, but the characteristics and availability of safe sites for tree regeneration in alpine ecosystems are not well understood. To better understand the potential of treeline ecotones to show infilling or upward shifts, we assessed microsite preferences of the conifers *Larix decidua*, *Pinus uncinata*, and *Pinus cembra* in upper treeline ecotones with different bedrock chemistry in the French Alps. At each of two sites on calcareous and two on siliceous bedrock, we compared microsites of 50 tree individuals to 50 randomly-selected reference microsites, considering substrate, ground cover, topography, and shelter proximity. In addition, we related these characteristics with the health of the individuals. We found that the three species were established in similar microsites, usually with some shelter. The occupied microsites reflected the available microsites in the area, but certain extreme microsite types remained unoccupied. Most individuals had a krummholz form or were bent, while only a small proportion presented signs of recent mechanical damage, desiccation, snow mold or herbivory, independent of microsite characteristics. Our study shows that the availability of safe sites unlikely limits the establishment of these conifers in the studied sites, suggesting that, instead, seed availability may be a major limitation for tree establishment in these alpine-treeline ecotones. Even in safe sites, the harsh alpine conditions limit the development of tree-species individuals into tree stature, but the strong recent length growth observed suggests favorable conditions for eventual tree expansion in and above current treeline ecotones.

Keywords Treeline ecotone · Establishment · Safe sites · Lithology · Health status · French Alps

Introduction

Seedling establishment constitutes one of the main filters in the dynamics of plant communities and is particularly crucial for the elevational advance of tree cover in and above the treeline ecotone (Lett and Dorrepaal 2018). Since such an advance implies a major biome shift, from a low-stature alpine to a forest ecosystem, understanding this filter in tree establishment is important for predicting future ecosystem services in mountains. A suitable microsite or safe site (*sensu* Harper 1977) is a place where the microenvironmental conditions increase the probabilities of germination, establishment and survival. Safe sites can be generated by microtopography (Rochefort and Peterson 1996), structures like rocks and/or deadwood (Johnson and Yeakley 2013; Resler et al. 2005), or vegetation (Germino et al. 2002). Therefore, a safe site may enhance establishment directly through physical protection, or indirectly through biotic interactions such as plant – plant facilitation (Wheeler

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et al. 2011). Although some advance has been made recently in characterizing suitable sites for tree establishment in treeline ecotones, no overall consensus has been reached about the importance and the nature of such sites, which may indeed depend on species-specific requirements as well as site-specific limitations (Caspersen and Sapruff 2005; Frei et al. 2018; Johnson and Yeakley 2016).

Shelter is frequently mentioned as favoring tree establishment in treeline ecotones, with rocks and deadwood being the main protective elements, enhancing survival through modification of wind speed, (UV) radiation, temperature, and soil moisture (Bürzle et al. 2018; Johnson and Yeakley 2019). Other mechanisms include accumulation of snow, increasing snowmelt water during summer and protecting against thermal fluctuations and against high-speed winds in winter (Germino and Smith 1999), although extended snow cover can also reduce survival due to snow mold infections and a shorter growing season (Barbeito et al. 2012). Favorable effects of microsite shelter on the seedlings include enhanced carbon gain during the growing season (Holtmeier 2009) and avoidance of desiccation and/or mechanical damage (Brodersen et al. 2019).

The microsite requirements of a seedling are species-specific (Maher and Germino 2006; Körner 2012). For example, seedlings of some species, like *Nothofagus*, require complete shelter from frost and/or excess radiation (Wardle 1984), while others, like *Pinus sylvestris* L., need gaps in the canopy or open spaces to establish (Hättenschwiler and Körner 1995), and yet other species survive best under the cover of alpine vegetation (Smith et al. 2003) or in moss seedbeds (Wheeler et al. 2011).

In the French Alps, *Larix decidua* (L.) Mill., *Pinus uncinata* Ramond ex. DC., and *P. cembra* L. are the main treeline-forming species, together with *Picea abies* (L.) H.Karst. (Didier 2001; Petitcolas et al. 1997). Forests with different mixtures of these species are normally found on the north-facing slopes of valleys, while the south-facing slopes present larger gaps with pasture areas for cattle and other agricultural use (Ali et al. 2005; Barros et al. 2017). Previous studies in Pyrenean treeline ecotones reported that *P. uncinata* establishment is higher at sites with sparse vegetation, bare soil, or litter and close to protective elements such as low shrubs (*Rhododendron ferrugineum* L.), stones, and microtopographic shelters that may ameliorate microsite conditions and promote snow retention (Batllori et al. 2009). In contrast, seedlings of *L. decidua*, in the European Alps, seem to depend on open spaces and exposed mineral soil to establish (Lingua et al. 2008; Risch et al. 2003). This species is shade intolerant, and the presence of thick litter and/or herb layers can inhibit the establishment (Garbarino et al. 2010). Dynamics of *P. cembra*, on the other hand, is heavily modified by the behavior of the nutcracker

(*Nucifraga caryocatactes* L.), its main disperser. Nutcrackers preferentially hoard seeds at sites that are easy to locate and where seed perishability is low (Neuschulz et al. 2015), i.e. in convex topography, such as small ridges and rocky outcrops, generating clusters of seedlings at such sites, even if these sites may not be environmentally optimal for tree establishment due to low soil and snow-cover depths (Holtmeier 2009; Neuschulz et al. 2015; Sorensen et al. 2022).

Although mountain conifers have been well studied in the Alps and the general ecological preferences of the different species are known, it is not clear to what extent these preferences manifest into microsite preferences in the treeline ecotone, or how strongly these preferences dictate the establishment pattern of tree-species individuals. Under ameliorating conditions caused by global warming, certain microsites may still be preferred but may no longer be the only sites that are safe for establishment. Assuming an adequate supply of viable seeds, this could strongly accelerate the advance of the treeline ecotone, especially if newly established trees provide additional safe sites (e.g. by providing shade and shelter), creating a positive feedback between establishment and further establishment.

Both the nature and the importance of microsite preferences for treeline expansion are also likely to differ between sites, for example in dependence of bedrock type. Bedrock types and resulting soils are recognized as fundamental determinants of plant community composition and diversity, with the main distinction being made between calcareous and siliceous bedrocks (Virtanen et al. 2003). On the one hand, substrate chemistry determines which species are most competitive, with many species being adapted to either the more acidic siliceous or the more basic calcareous soils (Gensac 1990; Petitcolas et al. 1997). On the other hand, the higher solubility and resulting porosity of calcareous bedrock leads to quick water percolation and drier soils than those developed on more acidic rock types (Michalet et al. 2002; Nemer et al. 2021). These effects of lithology on water availability could imply a higher risk of drought stress in treeline ecotones on calcareous substrates (Holtmeier and Broll 2005) and thus a stronger affinity of trees to establish in relatively moist microsites within such ecotones.

The French Alps present a heterogeneous landscape, with a mix of calcareous and siliceous bedrock types (Petitcolas et al. 1997) and diverse human activities molding the distribution of mountain forest (Saulnier et al. 2017). Both factors may have contributed to the regional differences in the conifer species that compose the treeline ecotone. In such heterogeneous landscapes, variation in microsite preferences among tree species and among lithologies may result in large variation in treeline-ecotone dynamics and are thus important to understand.

In this context, the aims of this study were to 1- Evaluate differences in the microsites preferences of *L. decidua*, *P. uncinata* and *P. cembra* established in the upper part of the treeline ecotone at calcareous and siliceous sites in the Southern French Alps; 2- Relate signs of stress and disturbance in the individuals (including seedlings, saplings, and small-statured adults) of the tree species with microsite characteristics. We hypothesize that (a) Given the differences in substrate, the individuals at calcareous sites will show a stronger preference for microsites with enhanced soil moisture than those at the siliceous sites; (b) Each of the species will show a particular preference for microsites, with *L. decidua* established in open spaces with sparse vegetation, *P. uncinata* in more sheltered microsites, and *P. cembra* on convex land forms (due to nutcracker behavior); and (c) More signs of stress and disturbance will be found in unsheltered individuals, particularly on calcareous soils where water availability is more limiting. By studying the controls on tree establishment in dependence of tree species and geological context, this paper seeks to help interpret and predict patterns of treeline ecotone dynamics in European mountains.

Materials and methods

Study area

The Briançon area is located in the driest part of the Southern portion of the French Alps, receiving around 764 mm of

annual precipitation (Blanchard 1921; Rolland et al. 1998). The area is characterized by a climate with a Mediterranean influence, with dry and hot summers and wet autumns and winters (Rolland et al. 1998; Dupire et al. 2017). We selected four sites, two with calcareous and two with siliceous bedrock origin, using geological maps (<https://www.gissol.fr/donnees/carte-sur-le-geoportail-4789>) and aerial imagery of the terrain (google maps), selecting sites with the appropriate parent material at the upper treeline ecotone and with good accessibility. The sites with calcareous origin were located close to Col du Granon (Cal_1) and close to L'Aiguille Rouge (Cal_2). The sites with siliceous origin were located close to Col du Granon (Sil_1) and close to Lac Laramon (Sil_2) (Fig. 1a). The size and shape of the sampling area varied among sites, depending on the terrain and the spatial distribution of the tree individuals (Table 1). This non-random selection of sites was chosen to allow an assessment of local, rather than landscape-scale, microsite preferences. All sites were located on north-facing slopes.

Species

Larix decidua Mill. (Pinaceae)

L. decidua (the european larch) is a deciduous conifer, naturally distributed in the central and southern mountains of Europe, from 180 m a.s.l to treeline elevation (up to 2400 m a.s.l) (Da Ronch et al. 2016). This species is light-demanding and acts as an early-successional species at treeline elevation, colonizing open spaces and recently deglaciated

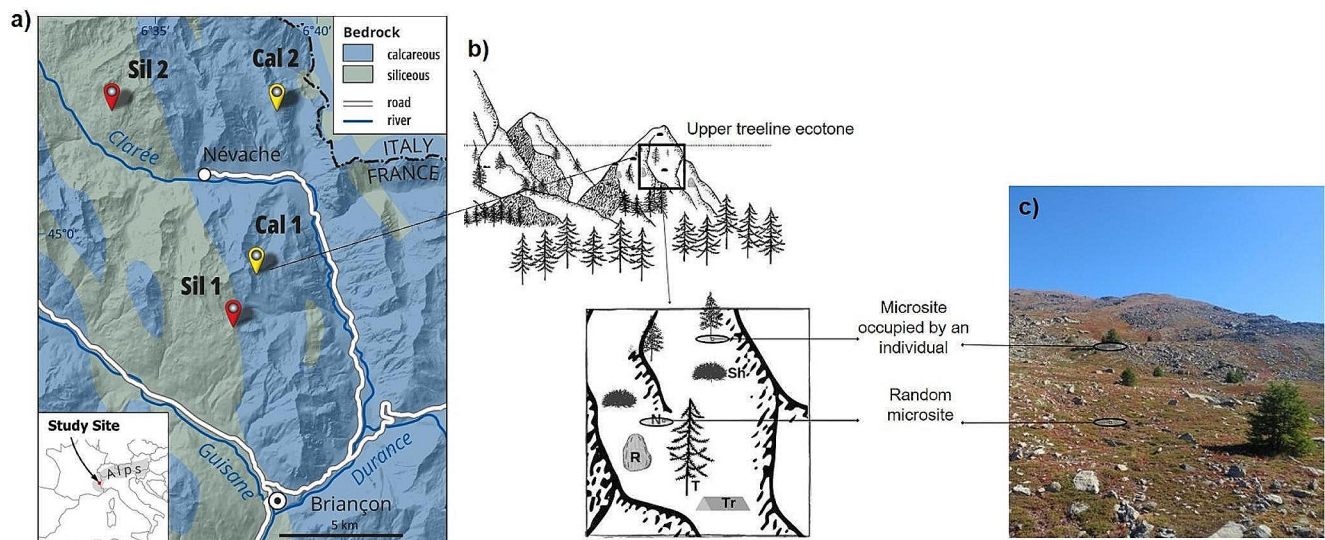


Fig. 1 (a) Location of the study sites in the Briançon area of the Southern French Alps, showing the lithology of the area. Map by Christiane Enderle. (b) Schematic illustration of a sampling site, including the main types of shelter (R=rock, Tr=terrace, Sh=shrub) and the position of the two types of microsites described. (c) View of one of the treeline ecotone sampling sites in the French Alps (Sil_2).

Photo: L.A Ramirez. Base map data: BD CARTO[®], Institut national de l'information géographique et forestière (IGN)- Downloaded from <https://geoservices.ign.fr/bdcarto> (last changed: 15/03/2023). Geology: Cartes géologiques vectorisées et harmonisées a 1/50,000 du BRGM, Service géologique national (BRGM) – Downloaded from <https://www.geocatalogue.fr> (last changed: 11/02/2020)

Table 1 Elevation range, coordinates of the center of the plot, surface area sampled, and number of individuals of the target tree species found within the sampling area at each treeline-ecotone site in the French Alps. Cal_1 and Cal_2 had calcareous bedrock and Sil_1 and Sil_2 had siliceous bedrock

Site	Elevation (m a.s.l.)	Coordinates	Area (m ²)	Number of individuals selected		
				<i>L. decidua</i>	<i>P. uncinata</i>	<i>P. cembra</i>
Cal_1	2380–2460	44°58′×53″N 6°37′×45″E	17,380	44	10	
Cal_2	2230–2300	45°02′×26″N 6°38′×37″E	20,602	8	43	
Sil_1	2410–2500	44°57′×45″N 6°36′×58″E	17,832	22	27	
Sil_2	2300–2450	45°02′×36″N 6°33′×30″E	20,656	18	2	34

terrains (Garbarino et al. 2010). It grows on a wide range of soils, from deep and well-structured to shallow stony, including calcareous soils (Caccianiga and Compostella 2012; Matras and Pâques 2008). It is the main treeline species in absence of *P. cembra* (Holtmeier 2009), but can appear mixed with it and other coniferous like *P. uncinata* and/or *P. abies* (Caccianiga et al. 2008).

Pinus uncinata Ramond ex DC. (Pinaceae)

P. uncinata (the mountain pine) is a shade-intolerant species, distributed in the Pyrenees, Western Alps and also in small populations in North-Eastern Spain (Balian et al. 2016). In the Pyrenees, where it has an extensive habitat, the species forms forests between 1700 and 2200 m a.s.l. but can reach elevations up to 2450 m a.s.l. in the treeline ecotone, depending on exposure and continentality (Holtmeier 2009; Batllori et al. 2010). The species is tolerant to low temperatures and grows mostly on moist soils, including peat-bog habitats (Holtmeier 2009). It is closely related to the krummholz form *P. mugo*, but its normal growth form is an upright tree.

Pinus cembra (L.) (Pinaceae)

P. cembra (the swiss stone pine) grows in the continental Alps, more frequently in the inner valleys up to 2500 m a.s.l., where it often forms the treeline (Caccianiga and Compostella 2012). It is described as a slow-growing species and weak competitor compared to other mountain tree species, but is well-adapted to the harsh climate in the subalpine belt. In early stages, it can tolerate shade, and germination can occur in organic or mineral soils and even on rocky surfaces. The seeds are dispersed over long distances exclusively by the nutcracker (*N. caryocatactes*). Its regeneration thus depends on the presence and behavior of this bird species (Ulber et al. 2004; Sorensen et al. 2022).

All three conifer species selected in this study are masting species with strong variation in the annual seed production

(Holtmeier 2009; Sorensen et al. 2022). This strategy, combined with the year-to-year variation in climatic conditions in these environments, may additionally limit establishment if most years do not coincide with favorable weather conditions for establishment.

Sampling methods

At each site, we located the upper treeline ecotone and, starting from the uppermost tree-species individual found, we sampled all individuals in a polygon perpendicular to the slope, until reaching a number close to 50 individuals (the exact number varies due to availability, see sample size and polygons area in Table 1). We found *L. decidua* and *P. uncinata* at all sites, while *P. cembra* was present only at the site Sil_2, where, in contrast, only two individuals of *P. uncinata* were found. We included all individuals present in the area selected, their height ranging from 5 to 400 cm, but mostly < 200 cm. We purposely refer to these as individuals, since the usual terminology of seedling or sapling does not fit the size, age and shape of these treeline-ecotone individuals. Since the taller individuals had too much influence on their microsite to allow reconstructing the original establishment conditions, we describe the microsites only for individuals < 150 cm tall. We used 1-m diameter microplots, within which we described the substrate (percentage of rock, vegetation and bare ground), local ground cover (bare ground, rock, and the vegetation types: grass, *Juniperus*, *Vaccinium* and low vegetation, referring to small herbs and/or cushion plants), macrotopography, at the slope scale (ridge, flat, valley) and microtopography, at the microsite scale (concave, convex, flat), and, when present, the characteristic of the shelter: type, size, direction, and distance from the individual. We also measured the slope and exposition. Additionally, we selected and described 50 reference plots, also of 1 m in diameter, placed semi-randomly by throwing a rock from each plot to determine the next, as null model, i.e. representing the microsite types available (Fig. 1b). For all the individuals selected, we measured basic biometrics:

height, age as estimated by counting the terminal internodes along the main stem (Batllori and Gutiérrez 2008), stem-base diameter (or diameter at breast height for individuals > 150 cm), length growth of main the shoot during the last two growing seasons (for individuals < 150 cm), health status (healthy, with mechanical damage, presence of snow mold, signs of herbivory, dried and dead) and shape (krummholz, bent or straight). Each individual and random plot has GPS coordinates and elevation (See Fig. S1). Sampling was conducted during the last two weeks of September, 2021.

Data analysis

To visualize differences in microsite characteristics among sites and species, we employed a multivariate analysis approach, using Factor Analysis of Mixed Data (FAMD). This method allows a dimension reduction of datasets containing both quantitative and qualitative variables (Pagès 2002). We used it to characterize the microsite conditions based on the environmental variables and to compare these conditions between microsites occupied by an individual of the target species and random plots. We expected that if there was a microsite preference, tree-species individuals would occupy a smaller range of the values along some of the FAMD axes than random plots. We also expected that these restricted ranges may differ between siliceous and calcareous sites and between the three species. For this analysis we selected the following variables: Qualitative variables: ground cover types, microtopography, macrotopography and shelter presence. Quantitative variables: vegetation cover (%), rock cover (%), bare ground cover (%), slope (°), eastness (sine transformation of aspect) and northness (cosine transformation of aspect). To avoid artifacts generated by missing data on shelter characteristics where no shelter was present, we excluded the variables related to shelter characteristics. We ran a FAMD of all sites combined to detect general differences in the microsite availability among sites, and separately for each site to detect microsite preferences (contrasting occupied microsites with the random plots within the sites). Since there were only 2 individuals of *P. uncinata* in the site Sil_2, we excluded this species in the analysis for that site.

We also analyzed the proportion of microsites with a particular type of shelter and type of ground cover for the three target species and the random plots, merging the data of all sites, and site by site, using Chi square analysis to evaluate statistical differences between microsites inhabited by a tree-species individual and the random plots.

Additionally, following the protocol proposed by Smith and Warren (2019), we modeled the probability of occupancy by an individual at a certain microsite, separating

the data by species and site. Since the response variable is binary (presence or absence of the individual) we used a Bernoulli or logistic GLM for modeling the data. For the full model we used the following variables: shelter size (log), shelter eastness (sine transformation of shelter direction) and shelter northness (cosine transformation of shelter direction), shelter distance (cm), shelter type, ground cover type, rock (%), bare ground (%), slope and microtopography type. We also included the most plausible interactions: shelter size (log) x shelter distance, northness x slope, eastness x slope, slope x microtopography, shelter size (log) x shelter type. Since the sample sizes varied among sites, we decided to concentrate on those sites with at least 40 individuals of the same species: *L. decidua* in Cal_1 and *P. uncinata* in Cal_2. We fit multiple models using all possible combinations of the predictors and their interactions, and then compared and selected the best model using the Akaike Information Criterion (AIC). We then performed a post hoc analysis, analyzing differences between levels of the factors included in the model, using Tukey Contrast for multiple comparisons of means.

To test our third hypothesis, i.e. more signs of stress and disturbance will be found in unsheltered individuals, we compared the health and shape of individuals established in sheltered and non-sheltered microsites using a Chi-square analysis and the length growth of the current year through a two-way ANOVA, using species and shelter as fixed factors.

All analyses were performed with the software environment R (version 2.3.5; R Core Team 2015), using the following packages *FactoMineR* (Lê et al. 2008), *factoextra* (Kassambara and Mundt 2017), *ggplot2* (Wickham 2011), *stats* (R Core Team 2015), *lme4* (Bates et al. 2009), *multcomp* (Hothorn et al. 2016), and *cowplot* (Wilke 2024).

Results

Differences in the microsite preferences among sites and species

Similar types of microsites were found at all sites, regardless of bedrock origin. In the graph of the first two dimensions of the FAMD, the point clouds of the sites were almost completely superposed, with most of the plots in the middle of the multivariate space (Fig. S2). This suggest that the available microsites are comparable between sites.

When analyzed by site, random plots were also superposed with those plots occupied by the target species in all of the sites (Figs. 2 and 3). However, the most extreme positions along the first two axes were mostly occupied by the random plots, possibly indicating a filter against extreme positions for tree-species establishment. For example,

strongly convex microsites (in Cal_1, Fig. 2), rocky microsites or microsites covered with *Juniperus* shrubs and/or located in valleys (in Sil_1 and Sil_2, Fig. 3) were not occupied by tree individuals.

Most microsites had some shelter, typically rocks, followed by terraces and shrubs. These trends were consistent across the four sites and did not differ between tree species or between occupied and random plots (Fig. S3, Table S1). Ground-cover types' frequencies also did not differ between occupied and random plots (Fig. S4, Table S1). In calcareous sites, the main ground cover types were low vegetation, rocks, and bare ground, while in siliceous sites patches of *Vaccinium* appeared consistently in all microsites, and patches of *Juniperus* were present mainly in the random plots (Fig. S4).

P. uncinata occupancy was modeled at the site Cal_2 ($N=43$). This occupancy was best predicted using the variables: *ground cover*, *slope*, and *microtopography* (Fig. 4a, b and c, Table S2, Table S3). There are higher probabilities of occupancy in sites with low vegetation and bare ground compared with rocks as ground cover. Occupancy is more likely on gentle than steep slopes and in concave than on convex or flat microsites (Table S3). This model explained 19% of the total variation (Table S2).

Occupancy of a microsite by *L. decidua* at the site Cal_1 ($N=44$) was best predicted using the variables: *ground cover* and *slope* (Fig. 4d and e; Table S2, Table S4). There are greater probabilities of occupancy in sites with bare ground and gentle slopes compared with sites with rocks and steep slopes. However, this model explained just 12% of the total variation (Table S2).

Biotic responses of individuals established in sheltered and non-sheltered microsites

In general, most of the individuals of the three target species were healthy, with only a small proportion showing signs of recent drought, mechanical damage, and/or snow mold. Very few individuals presented signs of herbivory (Fig. S5). No statistical differences were detected between the health status of individuals established in microsites with or without shelter (Fig. S5, Chi square and p values in Table S5). Overall, most of the individuals presented a krummholz shape, while a small proportion was straight or bent. These proportions were similar among species and with and without shelter (Fig. S6). Most of the individuals selected in this study were under 150 cm in height, but a few individuals reached heights of about 3 m. The length of the first internode of the leading branches varied from < 1 to 37 cm (Fig. S8). The age of most individuals was estimated to be less than 30 years (Fig. S7). The relationship between age and

height as well as the current-year length growth were independent of the presence of shelter (Tables S6 and S7).

Discussion

Our results show that (1) There are no clear differences in the microsite preferences of the three target species between sites with calcareous or siliceous bedrock, (2) The three tree species are established in similar microsites, and microsite preferences are not strongly expressed, with many of the random plots appearing to be also suitable for tree-seedling establishment. (3) There are no major differences in the health status, growth or shape between individuals established in sheltered or non-sheltered microsites. Since safe-site availability in the study sites is high, the main limitation for tree-species establishment in these ecotones is likely seed availability combined with generally low germination and survival rates. In particular, we expect a critical role for the timing of years with high seed availability and years with favorable conditions for establishment, which are less likely to align in mast-seeding species and in marginal climatic conditions like in treeline ecotones.

Differences in the microhabitat preferences among sites

In the European Alps, previous studies have shown that calcareous and siliceous sites not only differ in substrate chemistry, but also form specific and contrasting geomorphologies (Nemer et al. 2021; Nicklas et al. 2021). The chemical and physical differences lead to drastic differences in the plant communities (Michalet et al. 2002). Surprisingly, and contrary to our initial hypothesis, we found similar microsite preferences of tree individuals in calcareous and siliceous treeline-ecotone sites.

The treeline ecotone is a spatial phenomenon (Holtmeier and Broll 2005). Macroclimate controls treeline dynamics at regional and global scales, while variation in geomorphology and/or substrate modifies ecotone dynamics at landscape and hillslope scales, through the resulting heterogeneity in soils, hydrology, microclimate, and disturbance type and frequency (Malanson et al. 2007). The smaller the grain of observation, the greater the heterogeneity and the role of stochasticity, making patterns more difficult to interpret. Nevertheless, the effort is worthwhile as this is the scale at which many ecological processes take place (Bader et al. 2020). In our study, we assessed the microsite preferences of three tree-forming species at a very local scale. At this scale, the availability of potentially good microsites appears to be high, and there are no differences between sites of different geological origin. However, at larger scales, tree individuals

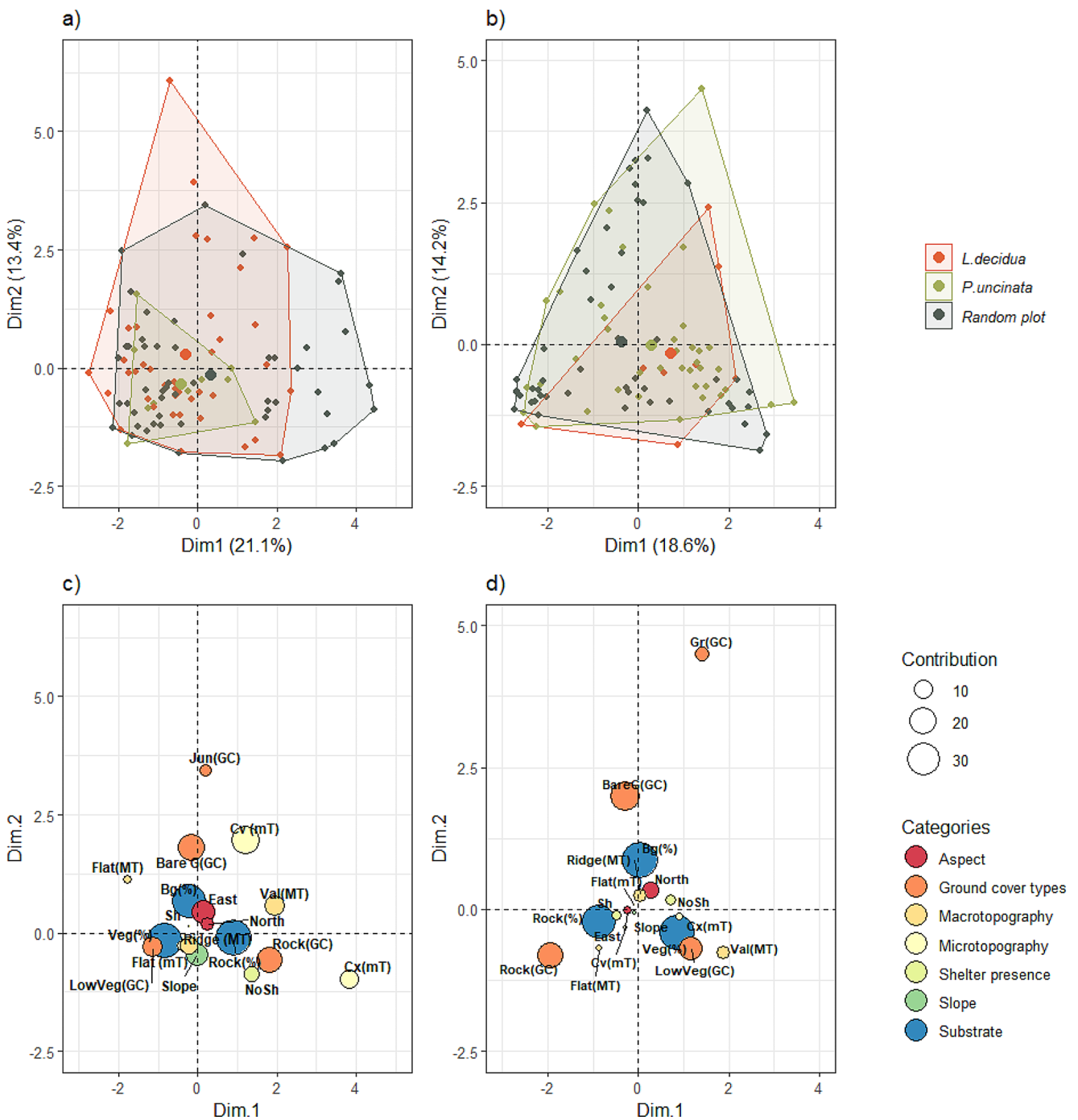


Fig. 2 Factor analysis of mixed data (FAMD) of all the microsites occupied by *Larix decidua* (orange) or *Pinus uncinata* (green) and the random plots (grey) at the upper treeline ecotone in the calcareous sites Cal 1 (a) and Cal 2 (b) in the French Alps. The contributions (in %) of the factor levels of different variables (colours) within the first two dimensions are expressed by the size of the bubble (c and d). Variables: aspect: sine transformation of the aspect (East), cosine

transformation of the aspect (North); ground cover types: bare ground (BareG (GC)), grass (Gr (GC)), *Juniperus* (Jun (GC)), low vegetation (LowVeg (GC)), *Vaccinium* (Vac (GC)); macrotopography: ridge (Ridge (MT)), flat (Flat (MT)), valley (Val (MT)); microtopography: concave (Cv (mT)), convex (Cx (mT)), flat (Flat (mT)); shelter presence: shelter (Sh), no shelter (NoSh); substrate: vegetation (Veg (%)), rock (Rock (%)), bare ground (Bg (%))

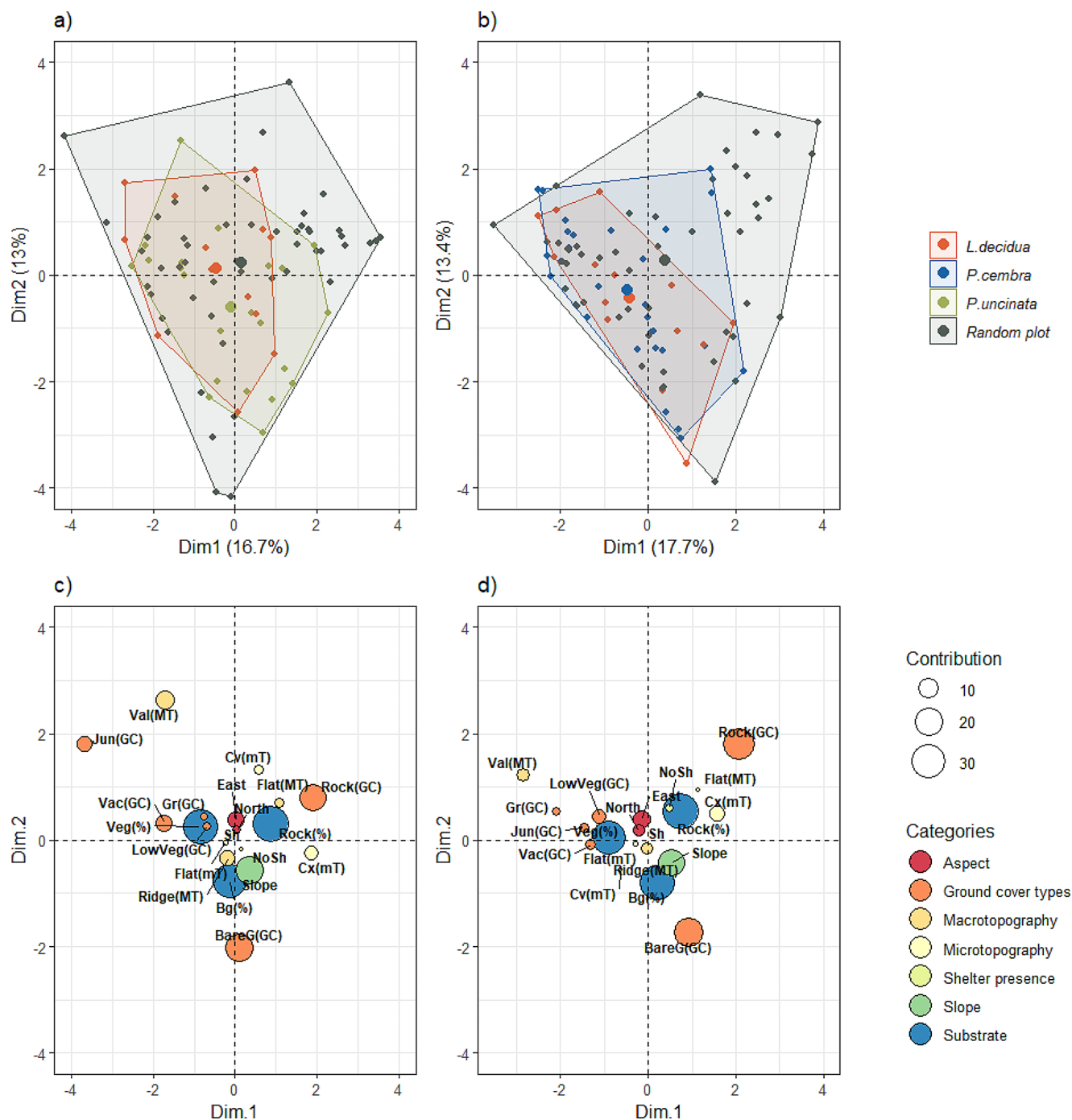


Fig. 3 Factor analysis of mixed data (FAMD) of all the microsites occupied by *Larix decidua* (orange), *Pinus uncinata* (green) (only in Sil 1) or *Pinus cembra* (blue) (only in Sil 2) and the random plots (grey) at the upper treeline ecotone in the sicileous sites Sil 1 (a) and Sil

2 (b) in the French Alps. The contributions (in %) of the factor levels of different variables (colours) within the first two dimensions are expressed by the size of the bubble (c and d). See Fig. 2 for variables abbreviations

were not randomly distributed but were restricted to certain landscape positions. This was most evident at the calcareous site Cal_1, where individuals were growing in an area at the base of a scree slope characterized by the presence of many large boulders, whereas no individuals were established on either the scree slope itself or the boulder-free

meadow adjacent to the site (Supplementary Fig. 1d). Thus, at a landscape scale, it appears that tree establishment is favoured in areas with high rock cover, which may provide microclimatic benefits (e.g. radiative warming from boulders, protection from snow and shelter from wind) as well as protection from livestock grazing. These correlated benefits

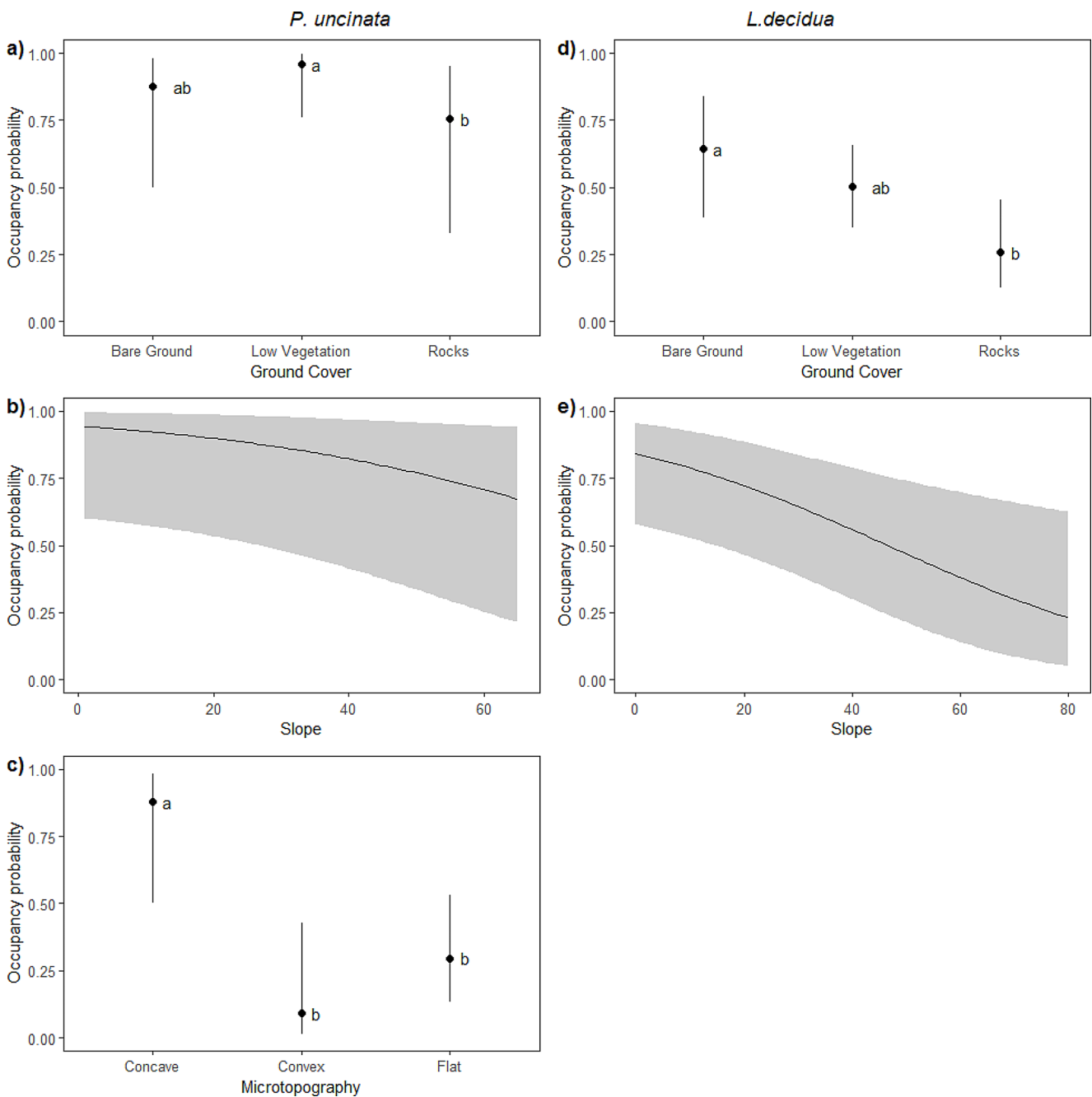


Fig. 4 Predicted probability of the occupancy by an individual of *P. uncinata* in microsites at the calcareous site *Cal_2* as a function of ground cover (**a**), slope (**b**) and microtopography (**c**) and by an individual of *L. decidua* in microsites at the calcareous site *Cal_1*, as a function of ground cover (**d**) and slope (**e**) in the upper treeline ecotone

of rocky sites have long been controversial in the treeline literature, particularly for the Andes (e.g. Kessler 2002). We do not offer a solution to this debate here, but note that at small scales, boulders do not appear to be essential for tree-species establishment.

in the French Alps. The data were modeled with a Bernoulli GLM. Bars and shaded areas indicate 95% confidence intervals. Different letters indicate differences detected in the multiple comparisons of means using Tukey contrasts (Table S3 and S4)

Differences in microsite preferences among species

Various studies have shown that the early establishment of trees in the treeline ecotone requires favorable microsites (Resler et al. 2005; Brodersen et al. 2019; Johnson and Yeakley 2016) and that species differ in their requirements for these microsites (Maher and Germino 2006; Körner

2012). However, in our study no differences between the three tree species could be detected, in accordance with the overall weak microsite preferences. This does not imply, that the known ecological preferences of the species are no longer in place, but it may suggest that microsites availability does not restrict the establishment of the species within the current conditions in the treeline ecotones of the Alps. However, other factors like seed availability, low germination or survival rates potentially limit the recruitment of new trees of these species in the upper treeline ecotone.

On the other hand, we did find clear differences in the tree-species composition among the four sites. Although the replication of sites is too low to assign these differences to specific factors, we can discuss some factors that may explain these patterns. Although all three tree species studied are distributed on both calcareous and siliceous bedrocks (Matras and Pâques 2008; Gómez et al. 2003), we found *P. cembra* only at one of the siliceous sites. It has been described that *P. cembra* develops better in non-calcareous than in calcareous substrates (Petitcolas et al. 1997). However, due to the species' obligate mutualism with its disperser (Camaret et al. 1998; Neuschulz et al. 2018), its absence at the calcareous sites, as well as its spatial distribution at the site where it was found, could well be related to reasons other than the substrate (Ali et al. 2005). Apart from nutcracker behavior, historical anthropogenic influences also affect the distribution of this species, in part due to clearing and pasturing along its distribution area and also due to its highly-valued timber (Motta et al. 2006).

Plant- fungal interactions are another key biotic interaction influencing the establishment dynamics of *P. cembra* (Merges et al. 2020). On the one hand, the mutualism of the species with mycorrhizal fungi is obligatory. In fact, afforestation with this species can only be successful if the seedlings are previously inoculated with mycorrhiza (Rainer et al. 2015). However, in the Italian Alps, Mandolini et al. (2022) reported that the mycorrhizal community in young *P. cembra* trees was more similar to that found in seedlings of *L. decidua* and other conifers than to the more diverse communities in adult *P. cembra*. This means that mycorrhizal colonization of *P. cembra* seedling roots can occur when other treeline tree species are present at the site (Mandolini et al. 2022) and does not necessarily require conspecifics. On the other hand, negative interactions with microorganisms can also play a role, since seedling survival of *P. cembra* is reduced when the abundance of pathogenic snow fungi is high (Merges et al. 2020).

Performance in sheltered and non-sheltered microsites

The upper treeline ecotone presents harsh environmental conditions that are directly reflected in the growth form of trees (Holtmeier and Broll 2020). Signs of past winter desiccation, frost damage and strong winds are commonly found in the survivors at these elevations (Holtmeier 2009). Contrary to our expectations, most of the individuals we found were healthy and only a few showed signs of recent mechanical damage, herbivory or drought, suggesting that our study was preceded by a few years of relatively mild conditions at these sites. Furthermore, although a higher proportion of individuals found were established in a sheltered microsite, as expected given the high availability of shelter in the plots studied, we did not detect any differences between sheltered and non-sheltered individuals in terms of health, growth rates or height~age relationship (although our age estimation may have low accuracy given the method used). This again suggests relatively mild conditions in which shelter is not essential. Favorable conditions in recent years, not only for biomass maintenance and possibly survival, but also for growth, were also indicated by the considerable length of current and previous year's shoots in many of the individuals.

Conclusions

The maintenance and expansion of tree populations in upper treeline ecotones depend on the combination of viable-seed rain and successful germination and establishment, but only established trees really matter for the advance of the forest towards the climatic treeline (Körner 2012). Our study shows that there is a high availability of safe sites for the establishment of the three target species in the treeline ecotone, but many of these sites are unoccupied. Seed availability thus emerges as a potential limitation for the establishment of the studied tree species in the treeline ecotones. In addition, the density of tree-species individuals in the ecotone may remain low due to high seedling mortality. Stress and disturbance for small trees are both strong in these environments, as testified by the irregular growth forms and small stature relative to the ages of the tree-species individuals. The relatively low proportion of recent damage and high current-year growth rates observed suggest that the year of study and one or several previous years had been favorable for tree growth and survival. If such conditions continue, as is likely considering the current directional change of climate, the stock of established small tree individuals could provide a source of fast treeline advance, growing into tree size and rising the elevation of the uppermost tree-sized individuals.

Grown into tree-sized trees, they could provide additional shelter and seed sources for further tree establishment. Long-term monitoring of seed availability, new recruitment and the persistence of established individuals could give us a better understanding of the dynamics of this ecotone.

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Data availability The datasets generated during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors have no relevant financial or non-financial interests to disclose.

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