**Microclimate drives seed germination phenology in alpine plant communities.**

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**Abstract (max 250 words)**

Understanding the plasticity of seed germination timing is important to predict the resilience of plant communities to climatic changes, yet we know very little about the influence of alpine microclimates on germination phenology. Mirroring temperature data series recorded in alpine soils, we conducted a continuous one-year incubator experiment with in-situ collected seeds to investigate germination phenology in two extreme conditions: (1) warmer temperatures and no snow cover, (2) cooler temperatures and lengthy snow cover, representing fellfield and snowbed alpine microhabitats, respectively. We replicated the experiment in mediterranean (21 species) and temperate (38 species) alpine communities from the same biogeographical region, the Cantabrian Mountains (northern Spain). We used the results to define eight traits related to germination phenology and found strong differences between microclimatic conditions. In autumn, species germinated more and faster in fellfield conditions. The opposite trend was observed during winter and summer, with higher germination in snowbed conditions. Consistent response patterns were found in the two alpine communities (Consistent to intraspecific?) , but the mediterranean community had higher overall germination, specially before winter due to the lower seed dormancy. Our results suggest that a warming scenario with reduced snow cover and higher temperatures will lead to accelerated germination rates and anticipated germination phenology and will likely have negative effects on those species with strict cold germination requirements, especially in temperate alpine communities.

1. Introduction

The **alpine environment** is characterized by extreme weather conditions, short growing season and high topographic roughness (Körner 2021). These characteristics translate into micro-topographic differences in the snow-melting and temperature regimes (Körner 1999) which in turn determine local alpine plant diversity (Schöb 2009). Different microclimatic conditions likely modifies plant community assembly processes (Körner 2003) and favour the development of adaptations to specific microhabitats (Graham et al 2012).

**Microsites conditions** (fellfield vs snowbed ADD description) heterogeneity patches.

Concordantly recent studies found that functional variability in alpine areas is mainly related to topographic gradients (Choler 2005). Thus, seems logical to think that **regeneration traits** (Grubb 1977), like dormancy and germination requirements, may differ according to this micro-topographic gradient. Recent studies highlight the importance of integrating these regeneration traits in plant community ecology (Jiménez-Alfaro et al. 2016) and Hoyle et al. (2015) found that germination strategies add an independent axis of variation relative to vegetation traits. However, the few studies that take into account microclimatic variability found no differing traits between fellfield sloper and sowbed communities (Shimono and Kudo 2005, Sergey??).

This seemingly contradictory results could be explained by the fact que we don’t fully understand the underlying causes of the variation observed and the complexity of processes involved in regeneration traits.

“Calcareous habitats are usually nutrient rich soils with alkaline pH (Hoffman et al 2016) and the presence of carbonates and small soil particles (Körner 2003), consequently they have low water holding capacity and drier soils than siliceous habitats (Körner 2003)”” In siliceous habitats the soils are nutrient poor and hace acidic PH increasing solubilitic of toxic compounds in the soil (Chapman 1965)”

**Germination** is a sensitive (Hoyle et al. 2015) and irreversible process that precedes the most vulnerable stages of plant life (REF), thus detecting the best possible conditions to initiate is key in order for plants to successfully regenerate (REF). Germination phenology and strategies have strong fitness implications (Donohue 2005) and it has been observed to be highly variable especially in alpine plant communities (Wagner and Simons 2009).

**Germination strategies** (DEFINITION) temperate vs Mediterranean. Globally, a period of cold stratification and warm cues are needed for temperate alpine species to initiate the germination process (Baskin and Baskin 2014, Fernández-Pascual 2020). In high mountains with Mediterranean climate it has been observed that most species are able to germinate immediately after dispersal if water is available but do not differ from temperate alpine species with improved germination after a period of cold stratification (Jiménez-Benavides 2005).

Originally, the move-along experiment was designed to determine which sequence of temperature were necessary for dormancy-breaking (Baskin & Baskin, 2003). The same authors (Baskin and Baskin 1998), found in previous studies that temperature was the main factor responsible for dormancy breaking (Also Körner 2021), therefore we design the study without water stress during growing season (i.e. when Tmean>2ºC).

“Experiments under conditions simulating the actual fluctuations in the field are needed in order to understand the habitat-specific germination strategy (Shimono and Kudo 2005)”.

Our approach to study germination phenology in alpine communities through a variety of calculated traits mimicked a whole year temperature regimes and snowcover period in two extreme microclimatic conditions.

Alpine grassland communities (threats)

The main goal is to study germination phenology/timing variation across microhabitat extreme regimes (snowbed vs fellfield) in alpine grasslands. Test these potential germination differences in 2 typical alpine communities (Temperate vs Mediterranean) in southern Europe. The specific questions we will answer in the study is how will microclimatic conditions modify the germination timing of alpine grassland species? Will alpine species from different communities show different patterns of germination? Is the alpine germination syndrome the more common strategy in Southern Europe alpine environments?

The novelty of these study include

* highly precise/realistic temperature wise experiment, following 1 whole year of development.
* Few experiments to study microclimatic effects on germination traits at community level (2 communities).

In order to study germination timing/phenology we aim to calculate different germination traits/metrics to answer the following specific questions and predictions.

**H: Microclimate (Fellfield vs Snowbed) will have an effect on germination timing/phenology.**

Hypotheses. We hypothesise that microhabitat filters will drive differences in the germination traits of species and communities. However, within communities there will be a dispersion of germination timing traits to favour temporal niche partition, especially in more productive snow-patch communities with deeper soils. According to the co-existence theory of Grubb (1977), niche differentiation may be different between communities with higher versus lower resources and competition (e.g. fellfields versus snowbeds). (from proposal objective 3)

In Fellfield incubator we have higher temperatures, longer growing season and shorter winter period with below 0 temperatures (mimicking exposed areas, ridges with no snow). Thus, we expect to see faster germination rates (absolute and t50), higher final germination as well as in autumn and spring but lower or no germination during winter conditions. (see table 1 for details).

In Snowbed incubator, with lower temperatures, shorter growing season and longer winter period with constant 0 degrees and darkness (mimicking under snow-like conditions). Thus, we expect to see slower germination rates (absolute and t50), lower final germination as well as in autumn and spring germination, however we expect higher germination during summer, and higher germination during winter under snow-like conditions (see table 1 for details).

In the Mediterranean community we expect to find more species able to germinate at lower temperatures, with positive response to darkness and fast germination with water available (i.e autumn germination) (Mediterranean germination syndrome, Giménez Benavides 2005, 2018). Meaning high autumn and spring germination, low t50 and environmental heat sum.

In the Temperate community we expect to find more species germinating after cold stratification and warm cues (Alpine germination syndrome = physiological dormancy). Recent metaanalysis has found this syndrome to be the most common in strict alpine species (Fernández-Pascual et al., 2021). Meaning high summer germination, high t50 and environmental heat sum.

2. Methods

*2.1 Study area and field sampling*

Our study focuses on alpine grasslands above 1900 m a.s.l in the Cantabrian mountains, a mountain range running E-W through northern Spain with altitudes reaching 2500 m a.s.l. The Cantabrian mountains are considered a transitional biogeographical hub between Eurosiberian and Mediterranean regions (Loidi et al., 2015), influenced by mediterranean/continental climate in southern slopes and temperate/oceanic climate in northern slopes (Fig 1). The particularity of its geographic location facilitates the coexistence of a complex array of communities and species adapted to both climates. At fine scales, studies in alpine habitats found that topographic heterogeneity creates a mosaic of microclimatic conditions (Scherrer, D. and Körner, 2011) which in turn determine changes in compositional (Jiménez-Alfaro et al., 2014) and functional diversity (García-Gutiérrez et al., 2018). Regional alpine communities are dominated mostly by *Poaceae* and *Cyperaceae*, but they are also rich in Hemicryptophytes and Chamaephytes.

We established two study communities in the most contrasting conditions of Cantabrian alpine habitats (Fig 1): (1) SW Mediterranean grasslands in Valles de Omaña and Luna Biosphere Reserve; and (2) NE Temperate alpine grasslands Picos de Europa National Park (see more details in Table 1).

Table 1. Description of our two alpine communities with soil pH, mean soil temperature, growing days (defined here as days when mean temperature > 3 ºC), temperature annual range and dominant species values extracted from own field data. Precipitation values extracted from the closest weather stations to the study sites.

|  |  |  |
| --- | --- | --- |
| Community | Mediterranean | Temperate |
| Location (decimal coordinates) | 42.910731 / 42.891192˚ N - 6.043621 / - 6.107621˚ E | 43.168822 / 43.201078˚ N - 4.826706 / - 4.830672˚ E |
| Soil pH | 3.8 – 4.8 | 6.5 – 6.8 |
| Growing season length | April – October | May - October |
| Mean Soil Temperature | 8.08 ºC | 5.13 ºC |
| Temperature annual range | 21.77 ºC | 30.66 ºC |
| 12 - year average precipitation (mm) | 1058 mm (mean 2010-2022 from Barrios de Luna) | 3004.79 mm (Vega de Urriellu, mean 2008-2014). |
| Dominant species | *Festuca summilusitana* and *Luzula caespitosa* | *Carex sempervirens* and *Helianthemum urrielense* |

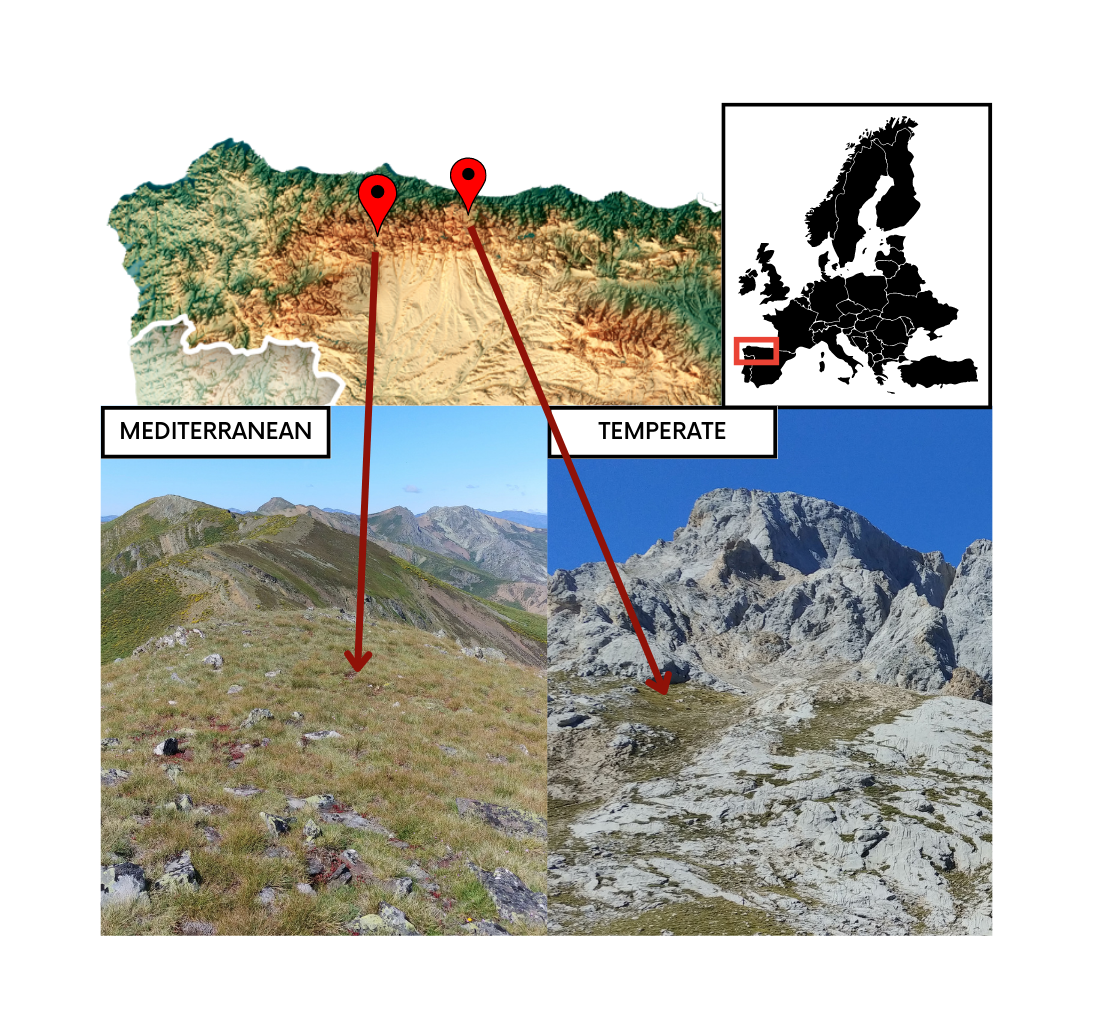


Fig 1. Map of the study sites in northwest Spain with the Cantabrian Mountain´s precipitation gradient? and the location of our two communities: Mediterranean and Temperate.

For each community we established 4 collection sites, treated as different populations, separated at least 500 m from each other. In each site, we recorded all vascular plant species co-occurring in a central representative plot of 3 m radius. The species recorded in the four sites were considered the target species pool in our experiment (22 sp. in the mediterranean and 49 sp. in the temperate communities). During August-September 2021 we visited the sites several times and gathered ripe seeds directly from the mother plants according to species maturity peak. Sampling took place within a 50 m radius from the central plot of each site and seeds were collected from at least 20 - 50 individuals chosen at random. The goal was to collect at least 200 seeds from each target species. In the mediterranean community we obtained enough seeds from 21 target species, covering two populations for each species except in 2 cases. In the temperate community we obtained seeds from 38 target species, from which 19 species were represented with 2 populations. Only three species (*Jurinea humilis*, *Thymus praecox* and *Silene ciliata*) were present in both communities (with two populations sampled in each community).

*2.2 Experimental setting*

We performed a continuous germination experiment to mimic one year of temperature regimes previously recorded in regional alpine communities. For this, we took 10 years of hourly soil temperatures data (2008-2019 field data collected with soil thermometers in our study area, M-Log5W, GeoPrecision, Ettlingen, Germany; accuracy: +/- 0.1 ºC at 0 ºC, resolution: 0.01 ºC) and transformed it into weekly maximum (T max) and minimum temperature (T min). We also used the registered temperatures to calculate the number of days with snow cover, estimated as days with no temperature variation between night and day (Zhang et al., 2005). Then, we chose the two extreme regimes in snow cover period and temperatures to recreate the same conditions in incubation chambers. The final experimental programs consisted of weekly temperature regimes (fig 2) with monthly photoperiod variation. Each experimental program was configured using Fitolog 9000 software (version 9308, Aralab Pharmaceutical Stability software) in Aralab incubators (Aralab climatic chamber Fitoclima S600 PL, equipped with 4 led modules 11W 350mA at 20%). This software allowed us to use ramp settings for gradual temperature changes along each day and to monitor the incubators remotely. Both incubators run simultaneously from July 2021 to September 2022. The experiment with warmer temperatures and no snow period will be referred as “fellfield incubator” and the experiment with cooler temperatures and long snow period program will be referred as “snowbed incubator”. Incubators remained closed during the whole winter period to avoid temperature changes.

Gráfico, Histograma

Descripción generada automáticamente

Fig 2. Weekly temperature programs configured in the incubators. Programs based on 10 years field data from Picos de Europa National Park (located within our study region). Both incubators were configured with weekly mean of maximum and minimum soil temperatures. Vertical lines mark germination timing traits calculated in our study. Horizontal lines represent the length of winter conditions (T mean < 3 ºC) in both fellfield and snowbed incubators. Additionally, photoperiod was modified every month according to our study region.

The two main differences between incubators were the temperature regimes and the length and climatic conditions during the winter period (defined as the period with T mean < 3 ºC). In the fellfield incubator we programmed winter with below 0 ºC temperatures with daily temperature and photoperiod fluctuation for 126 days while in snowbed incubator we programmed constant 0 ºC and darkness for 168 days (see winter period in Fig 2). Consequently, the growing season after winter also differed with 172 days in fellfield incubator and 122 days in snowbed incubator. Mean temperature differences between our two incubators during growing season was around three degrees Celsius each week (more detailed information about weekly programs in Appendix table Xx) reaching a maximum mean temperature of 18.5 ºC in fellfield incubator and 15.5 ºC in snowbed incubator, both during last week of August.

*2.3 Germination trials*

After field collection seeds were cleaned manually and sown within 20 days. We followed a sequential sowing according to species maturity peak in the field, meaning that the seeds were into the incubators in their optimum dispersal time. Each species population (N = 97) was placed in both incubators simultaneously, sowing four replicates of 25 seeds in 9 cm diameter Petri dishes (numbers were adapted for populations with fewer seeds available) with germination filter paper (Filtros Anoia S.A. paper for germination assays, Ref.518G085). Filter papers were kept soaked adding two or three millilitre of distilled water every two or three weeks and sealed with parafilm to maintain the moisture content, avoiding water stress throughout the growing season. During winter period incubators remained closed to avoid increases of temperature that could trigger seed germination and therefore no water could be added.

First sowing took place between weeks 36 and 41 of Julian calendar (except for 2 annual species sowed in week 31). By week 40 some species had already germinated more than 65% (specially from the mediterranean community) or were highly affected by fungus (N = 38 populations). Therefore, to keep track of germination timing across a full year we decided, only for these populations, to make a second sow from the same seed lot in week 42. Seed lots remained in room conditions since collection and during the whole experiment length. Since most alpine species require cold stratification and warm cues to germinate (Fernández-Pascual et al., 2021), we checked germination every two weeks before winter, and weekly after winter until the end of the experiment. As mentioned above, incubators remained closed during winter period thus no germination checks were performed. Seed were removed from the petri dishes once germination occurred (radicle > 1.5 mm long). Non-germinated seeds by the end of the experiment were cut open under the binocular loupe to visually assess if they were empty, infected, or looked normal. Seeds with white and firm embryos were considered viable (Baskin & Baskin, 2014). Empty or infected seeds were not included for further germination analysis. The experiment was terminated after 14 months (July 2021 - September 2022), for a total of 28 germination scorings. We removed from analysis populations with zero germination and those that had less than 25 % of viable seeds (assuming they were not able to break dormancy or the seed quality during collection was not optimal).

*2.4 Data analysis*

The final dataset used the raw scoring data of 54 species and 96 populations, representing 21 plant families. The total number of viable seeds used in the experiment was 16120.

We defined a set of traits related to germination timing (see Table 2). These traits were chosen to reflect the main germination phenology strategies and synthetise the patterns observed during one-year experiment. Germination rate and Total germination consider the whole raw data set germination scoring information; Autumn, Spring and Summer germination consider consecutive periods of time (calendar dates) while germination in Winter conditions takes into account the period with temperatures under specific threshold (T mean < 3 ªC), thus in this last case the length of the period between incubators differ (see Fig 2). Finally, t50 and Environmental heat sum focus on how much days and degrees the seeds experienced before reaching 50 % germination.

Table 2: Description of the functional germination metrics calculated in our study along with their ecological significance. Traits were calculated for petri dish and used as replicates per each population.

|  |  |  |
| --- | --- | --- |
| Germination traits | Description | Ecological significance |
| Germination rate | Cumulative germination by time passed (days). | Fast germination means more time to grow before winter but also higher vulnerability to early frosts. |
| Total germination | Total amount of seeds germinated at the end of the experiment (from 31/07/2021 to 19/09/2022). | Higher total germination means high potential of regeneration by seeds. |
| Autumn germination | Germination at mid-November (from 31/07/21 to 12/11/21. | Germination of species without physiological dormancy. Strategy to germinate fast and grow before winter ‘s adverse conditions. |
| Spring germination (relative) | Germination at mid-June relative to end of autumn (from 13/11/21 to 16/06/22. | Germination of species with cold stratification requirement but no need for high temperatures. Rapid onset of germination following snowmelt. |
| Summer germination (relative) | Germination at mid-September relative to spring (from 17/06/22 to 19/09/22. | Germination of species with cold stratification and warm cued germination. |
| Germination  in Winter conditions | Germination during winter period (from T mean ≤ 3 ºC until T mean > 3 ºC). | Germination of species able to germinate at very low temperatures (even under snow-like conditions) expanding their growing season and taking advantage of snowmelt water availability. |
| t50 | Time to reach 50% germination. Species under 50% germination were excluded from analysis (n = 72 populations). | Specific germination speed metric, highly comparable with other studies in the field. |
| Environmental heat sum | Sum of degrees (T mean) needed to reach t50. Species under 50% germination were excluded from analysis (n = 72 populations). | Number of degrees that species need to accumulate before germination. Strategy to avoid too early season germination after winter when frost events can still happen. |

We expect to have higher values of Total, Autumn and Spring germination as well as faster Germination rate in fellfield incubator, while we expect to have higher values of germination in Summer and during Winter Conditions as well as t50 in snowbed incubator. We don’t expect to find differences in environmental heat sum between incubators.

We performed the analysis of the raw data by fitting Markov Chain Monte Carlo generalized linear mixed models (MCMCglmm) with Bayesian estimation using the R package MCMCGLMM (Hadfield, 2010). To model germination timing traits, we used binomial MCMCglmms (family = multinomial2) while for time to reach 50% germination (t50) and environmental heat sum traits we scaled the values and used gaussian MCMCglmms (family = gaussian) (see table 2 for details). To calculate t50 trait in days, we fitted a linear model between the two scores dates before and after reaching 50% germination (1 week apart) to model the exact date and then count number days from sowing. Environmental heat sum was calculated summing the degrees experienced (considering daily mean temperature) from sowing date to t50 date for each petri dish.

Analysis was run separately for each community, models had incubator as fixed factor while phylogeny and population, nested within species, were specified as random factors. Phylogeny was included using a reconstructed tree for the 54 species, created with V.PHYLOMAKER R package (Jin & Qian, 2019). In all models we used weakly informative priors, with parameter-expanded priors for the random effects. Each model was run for 1 000 000 iterations, with an initial burn of 100 000 and a thinning interval of 100 (REF?). From the resulting posterior distributions, we calculated mean parameter estimates and 95% credible intervals (CI). To estimate phylogenetic signal of seed germination over all variables we used Pagels’s lambda (λ) (Pagel, 1999). Additionally, to investigate the patterns in our study area we run a more complex model to include both incubator and community as fixed factors with special interest on their interaction (see results in appendix table xx).

ADD synchrony (not significant) and MGT (not really used for tests but might be useful to quantify the specific rates)???

3. Results

Our study focused on germination phenology across one natural year, with detailed temperatures regimes to mimic extreme microclimatic natural conditions in two alpine communities from the same biogeographical area. For all the traits we found consistent germination patterns between the two alpine communities, however we also observed differences in the effect sizes of incubator and dormancy patterns.

The mediterranean community showed no significant differences between incubators for Total and Spring germination traits as well as for Environmental heat sum (see Fig 3A, left panel). However, we did find significant differences between incubators in the other traits: Germination rate (see Fig 2), Autumn germination were higher in fellfield incubator while Summer germination, germination in Winter conditions and t50 were higher in snowbed incubator (see Fig 3B left panel). The temperate community showed significant differences between incubators in all traits except in Environmental heat sum (see fig 3A, right panel). Germination rate, Total, Autumn and Spring germination were higher in fellfield incubator whereas Summer germination, germination in Winter conditions and t50 were higher in snowbed incubator (see fig 3B, right panel). We observed homologous responses for all our traits (although not all were significant) in both communities except for Spring germination. The effect size was always larger in the temperate community meaning that the differences between incubators were bigger.

Gráfico, Gráfico de barras, Gráfico de cajas y bigotes

Descripción generada automáticamente

Fig 3. Left panels for Mediterranean community and right panels for Temperate community. 3A. Shows effect of snowbed incubator in the eight traits for both communities, according to the MCMC-glmm analysis of raw data. Dots indicate mean effect size and whiskers are 95 % credible intervals (CI). The vertical dashed line marks zero effect, when the CI crosses the zero-line, means effect is not significant; positive numbers mean higher values in snowbed while negative numbers mean higher values in fellfield incubator. 3B. Mean value for the eight traits at each incubator for both communities. Errors bars in germination timing traits (Total, Autumn, Spring, Summer, Winter conditions) are binomial confident intervals. Errors bars in t50 and Environmental heat sum are standard error measures. Notice the different scales in y-axis in 3B.

Germination rate was analysed using the raw data from the whole experiment however to quantify it and facilitate the comparison here we also calculated the Mean Germination Rate (MGT, using GerminaR package in R) specific results per species can be consulted in appendix table XX. Germination rate was faster (p < 0.001) in fellfield incubator for both communities (Fig 4). In the mediterranean community MGT was 0.0236 in fellfield and 0.0181 in snowbed. Looking at the cumulative germination curves in Fig 4 there isn’t noticeable differences in rate before winter but after winter period we can observe a significant slower rate and delay in snowbed incubator (p < 0.001). In the temperate community MGR was 0.0123 in fellfield and 0.00484 in snowbed. We can already see a lower germination rate and delay in snowbed incubator before winter (see Fig 4) which is maintained after winter (p < 0.001).

Dormancy patterns observed did differ between communities across the experiment. In the mediterranean community, 82 % of species did not have dormant seeds and were able to germinate fast, reaching 0.45 – 0.55 of germination proportion before winter, after winter period germination peaked almost immediately even at low temperatures. In the temperate community only 55 % of species, with lower values of germination (0.05 – 0.25), were able to germinate before winter, indicating a higher degree of dormancy and after winter period germination peaked once temperatures surpassed 10 ºC.

Statistically significant lambda values (M=0.37, T=0.75) and intraspecific random factor (M= 2.19, T=1.63) for both communities, phylogeny was only significant in temperate community (7.53).

Gráfico, Gráfico de líneas

Descripción generada automáticamente

Fig 4. Cumulative germination curves, sum of all germination from each community, throughout the experiment. Based 22 species for the mediterranean community (left panel) and 38 species for temperate community (right panel). Within each panel, orange curve represents germination proportion in fellfield incubator and blue curve snowbed incubator. Flat areas represent winter period when no germination scores were done.

Overall, warmer temperatures and longer growing season of fellfield incubator promoted higher Total germination proportion. In the mediterranean community differences were existent but not statistically significant (Fig 3A, upper left panel), reaching 0.85 and 0.82 of germination proportion for fellfield and snowbed incubators respectively (Fig 3B, upper left panel). In the Temperate community incubator differences were statistically significant (Fig 3A, upper right panel), reaching 0.72 and 0.65 of germination proportion for fellfield and incubators snowbed respectively (Fig 3B, upper right panel). In Autumn germination we observed a also positive effect of fellfield incubator, reaching significantly higher germination values in both communities (Fig 3A second row). In the mediterranean community we registered a 0.55 germination proportion in fellfield compared to 0.4 in snowbed incubator and in the temperate community it was 0.24 of germination proportion in fellfield compared to 0.07 in snowbed. For Spring germination trait (relative to autumn germination), the mediterranean community showed no significant effect of incubator (Fig 3A, third row left panel) with both incubators reaching similar levels of germination proportion (0.28 and 0.3 in fellfield and snowbed, respectively, see Fig 3B third row left panel). On the other hand, for the temperate community, there is significantly higher germination in fellfield incubator with a germination proportion of 0.4 almost doubling snowbed incubator (Fig 3A and 3B, right panel third row). In contrast, Summer germination reached higher germination values in snowbed incubator (Fig 3A and Fig 3B fourth row) in both communities. In the mediterranean community reached values of 0.12 germination proportion compared to 0.03 in fellfield incubator. In the temperate community, differences between incubators were more noticeable, snowbed incubator reached 0.37 germination proportion compared to 0.08 in fellfield incubator. Germination in Winter conditions was noticeable higher in snowbed incubator for both communities (Fig 3 fifth row). Constant 0 º C and darkness seemed to favour germination with 0.2 germination proportion in the mediterranean community and 0.14 in the temperate community, in both cases five times higher than their respective values in fellfield incubator (0.04 and 0.02 for mediterranean and temperate respectively). When looking into t50 trait, we found significant higher values in snowbed incubator (Fig 3 sixth row), meaning that it took more time to reach 50% germination in the cooler incubator (snowbed). The average t50 time was 83 and 122 days in the mediterranean community and 164 and 236 in the temperate community for fellfield and snowbed incubator respectively (Fig 3B sixth row). To conclude with the traits, we found Environmental heat sum trait was not significantly different in neither of the communities. In the mediterranean community we calculated a mean value of 304 and 238 degrees Celsius in fellfield and snowbed respectively, while in the temperate community we calculated a mean value of 445 and 427 degrees Celsius in fellfield and snowbed incubator respectively (Fig 3B last row).

Additionally, we calculated synchrony index from GerminaR package. In the mediterranean community values were 0.539 and 0.558 for fellfied and snowbed, respectively while in the temperate community values were 0.372 and 0.350 for for fellfied and snowbed, respectively. Nevertheless, no significant differences were detected between incubators in either community and neither between communities in the complex model.

Finally, when testing differences between communities with the complex model (incubator and community as fixed factors), we found that the temperate community had a significant lower germination rate, lower autumn germination and bigger t50. The rest of the traits did not statistically differ between communities. Nevertheless, a significant interaction term in germination rate, autumn, spring, summer germination, t50 and environmental heat sum confirmed that germination differences between incubators were bigger in the temperate community for all traits (see detailed results from complex model in appendix table xx) as we can also visually assess in the Fig.3B.

Effects of random factors

Lambda signal (Pagel, 1999) was significant for all germination traits in both communities except for t50 not significant in the mediterranean community and environmental heat sum was not significant in either of the communities (see appendix table xxx).

Phylogeny was a significant factor for half of the traits (in mediterraenan community for Total, Spring, Summer and in Winter conditions germination; in temperate for Germination rate, Total and Summer germination and t50) and for those had a bigger effect than population. Intraspecific variation (population:species), had a significant effect in all traits for the mediterranean community and in the temperate community significant for all except for total germination and summer germination (see details in appendix table xxx).



*4. Discussion*

“we present a novel assessment of seed germination traits”

“Our approach mimicked realistic temperatures in situ for a whole natural year”

We found significant differences in several of the germination phenology/timing traits between incubators (microclimatic conditions).

Fellfield incubator, with warmer temperatures, longer growing season, and shorter winter period with below 0 degrees; had significantly faster Germination rate in both communities, higher Total germination significant only in temperate community, higher Autumn germination in both communities, significantly higher Spring germination only in the temperate community and higher environmental heat sum only in Mediterranean community.

Snowbed incubator, with cooler temperatures, shorter growing season and longer winter period at 0ºC and darkness; had significantly higher summer germination in both communities, higher germination in winter conditions and higher t50.

This combination of patterns in our results showed that there is a significant delay between extremes microhabitats in alpine areas, suggesting a temporal niche partition in germination related traits. More species are able to germinate in fellfield conditions and to do so earlier in the season (autumn and spring) however species with strict cold stratification and warm cue requirement can only germinate later in the season (summer). Concordantly, t50 values were higher in snowbed conditions.

A separate, worth-while point is that in winter conditions 0 degrees and constant darkness allowed higher germination than below 0 temperatures and daily photoperiod. This results suggest that under snow like conditions can alleviate and break dormancy of some species, able to start germination under snow, taking advantage of water availability during snowmelt and using the full extent of the growing season to developed seedlings able to survive topsoil desiccation during summer and also freezing events the following winter.

In particular,Temperate community seems to be more susceptible to timing variation probably because the germination requirements of most of their species are strict, with a strong prevalence of germination strategies according to their evolutionary history (phylogeny always significant random factor). In the temperate alpine community, most species required cold stratification (alleviating physiological dormancy, for long periods to undergo successful germination (corroborating the findings by Cavieres and Arroyo 2000, Hoyle 2015, Mondoni 2009, Shimono and Kudo 2005 and Schwienbacher 2011). Therefore, climate warming happening faster above the tree line (Kullman 2004 and Körner 2023) might be a greater threat to those species that will be restricted to more remote/shadowed/cool microsites where snow can last longer in contrast of species more adapted to fellfield conditions that will find their area expanded under current climate warming and will be able to germinate better.

The Mediterranean community responded otherwise, while temporal niche partition was still existing a higher proportion of species were non-dormant, able to germinate immediately after dispersal at both microclimatic conditions (This behaviour have been also categorized as opportunistic by Hoyle 2015, that can provide selective advantage if seedling mortality is low, some cases found by Mondoni ()) and under a wide range of temperatures (Corroborating Giménez-Benavides 2005 and 2018). In this situation spring germination may shift into autumn germination with major implications for species currently adapted to different timing (Mondoni 2012). Although literature agrees that temperature is the main factor influencing germination (Körner 2023, Baskin and Baskin 2014); the results of the Mediterranean community in our experiment, suggests that water availability may potentially have a stronger influence in germination than lack of high temperatures with species able to germinate at 9/6 ºC maximum when water was available.

Our two communities do not only differ in their climate but also in the bedrock (Mediterranean = siliceous, temperate= calcareous) which might partly counteract the water holding capacity of the community. Mediterranean climate is drier (especially in summer) but siliceous soil has higher water holding capacity. Temperate climate is wetter but calcareous soil has lower water holding capacity. Recent study by Tudela-Isanta 2018 has shown that germination traits differed between siliceous and calcareous bedrock in the Alps (calcareous showed higher tb (i.e. need more warmth to germinate), low base water potential (i.e. able to germinate in drier conditions) and low FGP compared to siliceous habitat). Drier soils also have low thermal conductivity and therefore become warmer (Graham et al 2010). Species inhabiting warmer soils tends to have a higher Tb as they have adapted to need more heat as a cue to initiate germination (Dürr et al 2015) possibly to prevent germination at dispersal time (Tudela Isanta 2018), reducing the chances of seedling emergence before winter when mortality due to frost events is higher (Rosback and Poschold 2015, Fernández PAscual 2017). In Tudela-Isanta 2018 their siliceous habitats rarely suffer water restriction and their species had higher base water potential i.e. hardly germinate under low water potentials. In their results non-dormant seeds had a wider suitable temperature range for germination.

Some studies like Bernareggi 2016, found that experimental warming in pushed an early phenology state by more than a week and that higher incubation temperatures increased the total germination observed. However, their results also provided limited prove warmer parentals change germination/dormancy. Their results suggest that more species would be able to germinate at lower temperature (10ºC) should increase their autumn and after winter (early spring) germination. In our experimental setting fellfield incubator (warmer) reproduced the same behaviour with faster germination rate and more autumn /spring? (only in temperate community) Germination, while snowbed incubator exhibit slower rates and higher summer germination.

However, the higher values of germination in fellfield communities may or may not be advantageous because, more germination indeed increase the chances of regeneration but if it happens at once this episode is very vulnerable to extreme heat waves, drought or freezing events (REF). While having a postponed germination after winter can at least assure that there will be no drought during early spring (REF). 1 germination episode higher risk higher benefit/chances of regeneration, several events lower risks lower benefit. Also having a persistent soil seed bank with seed with from same species with different levels of dormancy can be beneficial to secure regeneration in the good years (Schwienbaccher 2010) and not bet everything to the next year after dispersal, which could be very dry or very cold.

All species with higher/faster germination under warmer temperature regime (fellfield) suggest that snowbed specialist are limited to snow refugia either because they need snow/cold to alleviate dormancy or because in fellfield areas they are outcompeted.

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