# Microclimate regulates seed germination timing in Temperate and Mediterranean alpine 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 climate change, yet we know very little about the influence of alpine microclimates on germination timing. Mirroring yearly temperature data series recorded in alpine soils, we conducted a continuous seasonal temperature experiment with fresh seeds to investigate germination timing in two contrasting climatic conditions: fellfield (warmer temperatures and no snow period) and snowbed (cooler temperatures and lengthy snow period). We replicated the experiment in incubator chambers for species sampled in Mediterranean (N = 21) and temperate (N = 38) alpine communities from the same biogeographical region in the Cantabrian Mountains (northern Spain). By defining eight traits related to germination timing, we found consistent effects of fellfield and snowbed conditions in the two communities. In autumn, species germinated more and faster in fellfield conditions, while the opposite trend was observed during winter and summer, with higher germination in snowbed conditions. These responses were consistent with the expected germination syndromes of both communities, with lower dormancy and higher autumn germination in Mediterranean rather than temperate community. Our results suggest that a warming scenario with reduced snow cover and higher temperatures will lead to accelerated germination rates and will likely have disrupting effects on species with strict cold germination requirements, especially in temperate alpine communities.

## 1. Introduction

The regeneration niche defined as the requirements for a successful replacement within communities and is of great importance for understanding community ecology (Grubb, 1977). Despite being overlooked for the past decades (REF), a current change in paradigm has renewed the interest and research focus on this complex and essential plant stage (REF). 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 regeneration strategies add an independent axis of variation relative to vegetation traits. One of the phases of plant regeneration is seed germination;a sensitive (Hoyle et al. 2015) and irreversible process that precedes one of the most vulnerable stages of plant life (REF), thus detecting the best possible conditions to initiate is key in order to successfully regenerate (REF). Therefore, germination strategies have strong fitness implications in plant development (Donohue 2005) and are primarily regulated by intrinsic physiological factors and extrinsic environmental factors (REF).

A perfect study site for contrasting environmental factors is the alpine biome, characterized by extreme weather conditions, short growing season and high topographic roughness (Körner 2021). These particularities also explain why this environment has been difficult to prospect and investigate. At fine scales topographic roughness translate into a mosaic of microclimatic conditions (Scherrer, D. and Körner, 2011), especially incident in the snow-melting and temperature regimes (Körner 1999). Different microclimatic conditions likely modify plant community assembly processes (Körner 2003) and favour the development of adaptations to a specific microhabitat (Graham et al 2012). Concordantly recent studies found that it can determine local alpine plant diversity (Schöb 2009), changes in compositional (Jiménez-Alfaro et al., 2014) and functional diversity (García-Gutiérrez et al., 2018). Thus, seems logical to think that regeneration traits (Grubb 1977), like dormancy and germination strategies, may differ according to this micro-topographic gradient.

Globally, it has been defined an alpine germination syndrome, characterized by the requirement of a period of cold stratification followed by warm temperatures to initiate the germination process (Baskin and Baskin 2014, Fernández-Pascual 2021) likely to avoid unfavourable periods and ensure germination at the beginning of growing season, when water is available and seedlings have time to develop a root system before summer dry periods (REF). A special case has been proposed for high mountains with Mediterranean climate where species are able to germinate immediately after dispersal if water is available, but they also show improved germination after a period of cold stratification, referred to as Mediterranean germination syndrome (Jiménez-Benavides 2005).

Even though these syndromes have been defined, germination strategies have been observed to be highly variable especially in alpine plant communities (Wagner and Simons 2009) and one of the potential causes are the mosaic of microclimatic conditions. However, the few studies that take into account microclimatic variability found no differing traits between fellfield sloper and snowbed communities (Shimono and Kudo 2005, Sergey??). Nevertheless, these studies rarely mimic real field conditions fluctuations and are “needed in order to understand the habitat-specific germination strategy (Shimono and Kudo 2005)”.

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.

Filling this knowledge gaps will help us understand and/or predict community responses to future climate scenarios.

### Present OUR study

The approach we present here is to study germination timing across a whole year in two contrasting microclimatic conditions measured in alpine grassland communities through a variety of germination traits. The study used highly precise and realistic temperature regimes and focused on an arrange of germination traits to answer specific questions at community level.

For the study purpose we described two contrasting microclimatic conditions: Micro- valleys (referred to as “snowbeds”) aka areas with dense plant cover, long snow cover and high moisture; and micro-edges (referred to as “fellfields”) aka more open and exposed areas subjected to freeze and thaw cycles without snow protections on winter and dry periods in summer.

### Questions and hypothesis

The specific questions we want to investigate are: (1) will microclimatic conditions modify the germination phenology of alpine communities? And (2) do alpine communities from different macroclimates show different patterns of germination phenology?

Regarding out first question, we hypothesise that microclimatic conditions will result in differences in the germination phenology of alpine communities. Fellfields have higher annual temperatures, longer growing seasons, shorter winters and more frequent freezing events than snowbeds (Table 1). Thus, we expect fellfield microclimates to result in faster germination, higher total germination and lower or no germination under snow. In snowbeds, we expect the opposite germination pattern. Considering our second question, we hypothesise that a temperate alpine community will show a higher concordance with the global alpine germination strategy (Fernández-Pascual et al. 2021) than a Mediterranean alpine community. In the temperate alpine community we expect more germination after cold stratification and under warmer germination temperatures, while in the Mediterranean community we expect faster germination and germination at colder temperatures (Giménez Benavides 2005, 2018).

## 2. Methods

### 2.1 Study system

Our study focuses on alpine grasslands between 1900 and 2500 m a.s.l in the Cantabrian Mountains, a mountain range running E-W in northern Spain. The Cantabrian mountains are considered a transitional biogeographical hub between Eurosiberian and Mediterranean regions (Jiménez-Alfaro et al., 2021), 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 with species adapted to both climates. Regional alpine communities are dominated mostly by *Poaceae* and *Cyperaceae*, but they are also rich in Hemicryptophytes and Chamaephytes.

### 2.1 Seed sampling

We established two study communities in two contrasting ecosystems of the Cantabrian high mountains (Fig 1): (1) Mediterranean high mountain grasslands in the Valles de Omaña and Luna Biosphere Reserve; and (2) temperate alpine grasslands in the Picos de Europa National Park (see more details in Table 1). Within each community we established four seed sampling sites, separated at least 500 m from each other, assuming they form the same meta-population~~., but we decided sample different subpopulations (referred to as “sites”) to maximize intraspecific variation.~~ 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 eight sites were considered the target community in our experiment (22 species in the Mediterranean and 49 species in the Temperate communities).

During August-September 2021 we visited the sites several times and sampled ripe seeds or fruits (hereafter ‘seeds’ for simplicity) directly from the mother plants of the target species. In general, we followed standard protocols for sampling seeds of wild populations to maximise intraspecific genetic diversity (https://www.publicgardens.org/resources/ensconet-seed-collecting-manual-wild-species). 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, by maximizing spatial sampling within the community. In the Mediterranean community we obtained enough seeds from 21 target species, covering two sites for each species except in 2 cases. In the Temperate community we obtained seeds from 38 target species, of which 19 were represented by 2 sites. Only three species (*Jurinea humilis*, *Thymus praecox* and *Silene ciliata*) were present in both communities (with two sites sampled in each community).

### 2.2 Microclimatic scenarios

We performed a continuous seasonal germination experiment to mimic one year of temperature regimes as previously recorded by us in the study communities. We used the temperate sites to establish our microclimatic scenarios because we had the longest temperature records (10 years vs. 1 year for the Mediterranean sites) and because the temperate system shows the highest microclimatic variation between snowbeds and fellfields. To create our microclimatic scenarios, we took 10 years of hourly temperature data (2008-2019 field data collected in alpine soils of our four sites at the Picos de Europa National Park, M-Log5W, GeoPrecision, Ettlingen, Germany; accuracy: +/- 0.1 ºC at 0 ºC, resolution: 0.01 ºC) and transformed it into weekly resolution for maximum (T max) and minimum daily temperatures (T min). We also used the registered temperatures to calculate the number of days with snow cover, estimated as days with less than one degree Celsius temperature variation around zero degrees between night and day (Zhang et al., 2005). Then, we chose the two most contrasting regimes in snow cover period and temperatures to recreate those same conditions in incubation chambers. The final experimental scenarios consisted of a weekly-resolution temperature programme (fig 2) with daily temperature ramps between daily Tmax and daily Tmin, and monthly-resolution photoperiod regimes. 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). 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 experimental chamber with warmer temperatures and no snow period mimicking micro-edge topography will be referred to as “fellfield incubator” and the chamber with cooler temperatures and long snow period program mimicking micro-valleys will be referred as “snowbed incubator”.

The major differences between incubators were the temperature regimes and the length and climatic conditions during the cold 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 the snowbed incubator we programmed constant 0 ºC and darkness for 168 days (see “cold period” in Fig 2). Consequently, the growing season after the cold period also differed, with 172 days in the fellfield incubator and 130 days in the snowbed incubator. Mean temperature differences between our two incubators during the growing season were 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 the fellfield incubator and 15.5 ºC in the snowbed incubator, both during last week of August.

### 2.3 Germination trials

Fresh seeds were manually cleaned and sown within 20 days of collection. We did a sequential sowing of each species and population according to their maturity peaks in the field, so seeds entered the incubators at their optimum dispersal time. Each seed lot (species x site, N = 97) was placed in both incubators simultaneously, sowing four replicates of 25 seeds in nine cm diameter Petri dishes (numbers were adapted for seed lots with fewer seeds available) with germination filter paper (Filtros Anoia S.A. paper for germination assays, Ref.518G085). Filter papers were kept soaked by adding two or three ml of distilled water every two weeks. Dishes were sealed with parafilm to maintain the moisture content, avoiding water stress throughout the growing season. During the cold period incubators remained closed to avoid increases of temperature that could trigger seed germination and therefore no water was added.

First sowing took place between weeks 36 and 41 of the 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 seed lots). Therefore, to keep track of germination timing across a full year we decided, only for these seed lots, to make a second sow of 50 extra seeds in week 42. Seed lots remained in room conditions between collection and sowing ~~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 the cold period thus no germination checks were performed. Seeds were removed from the petri dishes once germination occurred (radicle > 1.5 mm long). Non-germinated seeds at 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, i.e. potentially germinable (Baskin & Baskin, 2014). Empty or infected seeds were not considered for further analysis. The experiment was terminated after 14 months (July 2021 - September 2022), for a total of 28 germination scorings. We removed from further analysis populations with zero germination and those that had less than 25 % of viable seeds (N = XX), assuming we were not able to break dormancy or the seed quality during collection was not optimal. The final dataset (Appendix X) consisted of the raw scoring data for 96 seed lots of 54 species representing 21 plant families. A total of 16,120 viable seeds were used in the experiment.

### 2.4 Data analysis

We defined a set of metrics to describe germination timing (see Table 2). These metrics were chosen to synthetise the diverse patterns observed during the continuous germination experiment, by considering their functional ecological significance in the alpine ecosystem. Germination rate and Total germination consider the germination scores for the whole experiment. Autumn, Spring and Summer germination consider specific periods of time measured in calendar dates (i.e. equal length between incubators). Germination in cold conditions considers germination at the end of the winter period, defined by a specific thermal threshold (T mean < 3 ºC) (i.e. different length between incubators) (see Fig 2). We also calculated synchrony index and mean germination time using GerminaR package (Lozano-Isla et al., 2019). Finally, time to reach 50% germination (t50) and Environmental heat sum (EHS) focused on how much days and accumulated degrees the seeds experienced before reaching 50% germination.

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 the synchrony index, mean germination time, t50 and EHS 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 to model the exact date and then count the number days from sowing. EHS was calculated summing the degrees experienced (considering daily mean temperature) from sowing date to t50 date for each petri dish.

For each community, we computed the models with incubator as a fixed factor, while phylogeny and site (site 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 compare the patterns in our two communities we run a more complex model to include both incubator and community as fixed factors with special interest on their interaction.

## 3. Results

### 3.1. Community synthesis

Most traits from our study showed consistent patterns between the two alpine communities (fig 3B), however we did observed differences in their effect sizes (fig 3A).

In the Mediterranean community we found significant differences between incubators in most of the traits: Germination rate (see Fig 4) and Autumn germination were higher in fellfield incubator while Summer germination, Germination in cold conditions and t50 were higher in snowbed incubator (see Fig 3B left panel). Nevertheless, we found no significant differences between incubators for Total and Spring germination traits as well as for EHS (see Fig 3A, left panel). The Temperate community showed significant differences between incubators in all traits except in EHS (see fig 3A, right panel). Germination rate, Total, Autumn and Spring germination were higher in fellfield incubator whereas Summer germination, Germination in cold 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 observed between incubators were bigger.

### 3.2 Germination traits

#### Total germination

Overall, warmer temperatures and longer growing season of fellfield incubator promoted higher Total germination proportion. In the Mediterranean community differences existed but were 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).

#### Autumn germination

In Autumn germination we observed a 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.

#### Spring germination

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).

#### Summer germination

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.

#### Cold conditions germination

Germination in cold 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).

#### Environmental heat sum

We found EHS 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).

#### Synchrony index

The synchrony index calculated in the Mediterranean community showed mean values of 0.539 and 0.558 for fellfield and snowbed incubator respectively, while in the Temperate community values were 0.372 and 0.350 for fellfield and snowbed respectively. Nevertheless, no statistically significant differences were detected between incubators in either community or neither between communities.

### 3.3. Germination rate traits

Germination rate was analysed using the raw scoring germination data. Germination rate was found significantly faster (p < 0.001) in fellfield incubator for both communities (Fig 4). Looking at the cumulative germination curves in Fig 4, for the Mediterranean community (left panel), there was no noticeable differences in rate before cold period but after it we can observe a delay in germination in snowbed incubator (p < 0.001). In the Temperate community, however we already observed a lower germination rate and delay in snowbed incubator before winter (see Fig 4, right panel) which is maintained after winter (p < 0.001).

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 incubato. 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).

We also calculated the Mean Germination Rate (MGR) per each species and can be consulted in appendix table XX. In the Mediterranean community MGR considering all species was 0.0236 in fellfield and 0.0181 in snowbed. In the Temperate community MGR was 0.0123 in fellfield and 0.00484 in snowbed.

### 3.5. Community comparison

We also tested differences between communities with a more 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 the model for all traits indicate that germination differences between incubators were bigger in the Temperate community (see detailed results from complex model in appendix table xx).

### 3.6. Phylogeny and random factors

We found a strong phylogenetic signal, calculated by Pagel’s Lambda (Pagel, 1999), in both communities and with similar patterns. All germination timing traits had mean lambda values above 0.8 while traits related to germination speed (germination rate, t50 and EHS) showed values lower than 0.4 in the Mediterranean community and between 0.4 - 0.7 in the Temperate community (see appendix table xxx). The effect of random factors was also consistent between communities showing significant effects of phylogeny and intraspecific variability in all germination timing traits, although phylogeny always explained a higher proportion of variability (except the autumn germination in Mediterranean community). Germination speed traits in the Mediterranean community had no significant effect of phylogeny although there was a significant effect of intraspecific variation (see details in appendix table xxx).

## 4. Discussion

Our study focused on germination timing presents a novel assessment of seed germination traits across a whole year. Our approach mimicked real in-situ soil temperatures from contrasting microclimatic natural conditions in two alpine communities from the same biogeographical area.

We found significant differences in almost all germination timing traits between microclimatic conditions (micro-edges = fellfield, micro-valley = snowbed) and consistent patterns between communities.

In Fellfield incubator we found significantly faster Germination, higher Total germination, higher Autumn germination, significantly higher Spring germination. In Snowbed incubator we found significantly higher summer germination, higher germination in cold conditions and higher t50. No differences in heat environmental sum between incubators.

The Mediterranean community showed a consistent germination trait differentiation between microclimatic conditions except in total and spring germination and EHS. Although total germination at the end of the experiment did not statistically differ between incubators, values in fellfield were slightly higher. The cumulative germination curve showed that there is a significant germination delay between contrasting microclimatic conditions after the cold period. We found a higher proportion of non-dormant species (82%), able to germinate immediately after dispersal at both microclimatic conditions if water is available. Consequently, in Autumn germination we found the higher germination proportion throughout the year in both incubators. This behaviour of rapid germination after dispersal has been also categorized as opportunistic by Hoyle 2015 and can provide selective advantage if seeds achieve ripe and shed “early in the season” and therefore seedling have enough time to develop, and mortality is low during the cold period (some cases found by Mondoni () and under a wide range of temperatures. Corroborating Giménez-Benavides 2005 and 2018. Germination in spring (relative to end of autumn) did not differ between microclimatic conditions suggesting that species from the community are equally adapted to germinate in early season, either under snow-like conditions or at very low temperatures. Species able to start germination under snow are taking advantage of water availability during snowmelt and can use the full extent of the growing season to develop seedlings leaves and roots able to survive topsoil desiccation during summer and freezing events the following winter (REF). At the end of the growing season (summer germination), most species in fellfield conditions were already germinated thus we observed a significantly higher germination in snowbed conditions, probably germination of species that have cold stratification (alleviated during the cold period) and higher temperature requirements (achieved later in the season). Consequently, we observed a significantly higher t50 in snowbed incubator and not statistically different EHS, meaning that species germinated when they achieved/accumulated that thermal threshold/requirements although in snowbed incubator take longer to achieve. The phylogenetic signal, measured as Pagel’s lambda, showed values above 0.8 in germination timing traits (total, autumn, spring, summer, cold conditions) suggesting that are phylogenetic conservative traits. However, traits related to gemination speed (germination rate, t50 and EHS) showed below 0.4 values suggesting an evolutionary selection. The results of the random effects showed that intraspecific variation partly explained the variability observed between the incubators. Nevertheless, accordingly with Pagel’s lambda results, phylogeny explained a higher proportion of variability in germination timing traits.

The Temperate community showed a consistent trait differentiation in all germination traits except EHS. Total germination was found to be significantly higher in fellfield incubator, probably because the higher temperatures experienced in the incubator and although more germination indeed increases the chances of successful regeneration the timing and strategy of it is what in the end will determine if will be or not successful. The cumulative germination curve showed that there is a significant germination delay between microclimatic conditions from the beginning of the experiment. We observed a lower proportion on non-dormant species (55%) and germination in Autumn barely reached 0.25 and 0.05 germination proportion in fellfield and snowbed incubators, respectively, suggesting a strong prevalence of physiological dormancy. Germination in spring, was significantly higher in fellfield probably because the higher temperatures experienced in the incubator during that period that had alleviated both cold stratification and warm cues requirements, while snowbed species still did not reach that thermal threshold. Having a postponed germination after the cold period can at least assure that there will be no drought during early spring. The opposite trend was observed in summer germination, most species in fellfield conditions were already germinated thus we observed a significantly higher germination in snowbed conditions, after cold stratification took longer for species to reach temperature requirements. Consequently, we observed a significantly higher t50 in snowbed incubator and not statistically different EHS, meaning that species germinated when they achieved/accumulated that thermal threshold/requirements although in snowbed incubator take longer to achieve. The phylogenetic signal, measured as Pagel’s lambda, showed values above 0.75 in all germination traits except t50 and EHS, suggesting that most traits are phylogenetic conservative and t50 and EHS showed below 0.5 values suggesting an evolutionary selection. The results of the random effects showed that intraspecific variation partly explained the variability observed between the incubators in all traits except total and summer germination. Nevertheless, accordingly with Pagel’s lambda results, phylogeny explained a higher proportion of variability in all germination traits except t50 and EHS.

Another worth-while point, common in both communities, is that snowbed cold conditions (0ºC degrees and constant darkness) allowed higher germination than fellfield cold conditions (below 0ºC temperatures and daily photoperiod). These results suggest that under snow like conditions can alleviate and break dormancy of some species, while below 0ºC temperatures showed detrimental effects on germination. Species that live in micro-valleys (snowbed) can start germination under snow are taking advantage of water availability during snowmelt and using the full extent of the growing season to develop seedlings leaves and roots able to survive topsoil desiccation during summer and also freezing events the following winter. Species that live in micro-edges (fellfields) must be able to endure below 0 ºC temperatures and wait until temperatures surpasses at least 5ºC according to out experiment.

When we compare both communities even observing similar patterns, the Temperate community had a significant lower germination rate, lower autumn germination and bigger t50; and a significant interaction term between community and incubators indicate that germination differences between incubators were bigger in the Temperate community.

Our results confirm the differences between the germination alpine and mediterranean syndromes previously described (Giménez-Benavides, Basking and Basking, Fernández-Pascual 2021) alpine germination syndrome corroborating the findings by Cavieres and Arroyo 2000, Hoyle 2015, Mondoni 2009, Shimono and Kudo 2005 and Schwienbacher 2011). And also confirm that microclimatic conditions can be one of the reasons of the high variability of germination strategies in alpine habitats.

Future climate scenarios, with 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.

In these warming scenarios spring germination may shift into autumn germination if thermal requirements are met (Bernareggi 2016) and would have major implications for species currently adapted to different timing (Mondoni 2012). In our experimental setting fellfield incubator (warmer) reproduced the same behaviour with faster germination rate and more autumn /spring. 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 5ºC when water was available. 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)

Study limitations:

Our two communities do not only differ in their macroclimate but also in the bedrock (Mediterranean = siliceous, Temperate= calcareous) and a recent study by Tudela-Isanta 2018 has shown that germination traits differed between siliceous and calcareous bedrock in the Alps, potentially having a cofounding factor.

Syncrony, we found no differences on synchrony between incubators, the strategy seems to be mainly related to intrinsic factors, if it happens at once this episode is very vulnerable to extreme heat waves, drought or freezing events (REF) while having a more stretched germination period can help avoid this punctual detrimental factors. 1 germination episode higher risk higher benefit/chances of regeneration, several events lower risks lower benefit.

Study limitations

Conclusions + future directions!

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.

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# Tables and figures

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.

|  |  |  |
| --- | --- | --- |
|  | Mediterranean community | Temperate community |
| Location (center of four sampling sites per community) | 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 annual Temperature in the soil (5 cm depth) | 8.08 ºC | 5.13 ºC |
| Temperature annual range in the soil (absolute max – absolute min, BIO5 – BIO6), from Picos 10 years and from Omaña 1 year. | 21.77 ºC | 30.66 ºC |
| Annual precipitation (mm) from nearest station | 1058 mm (mean 2010-2022 from Barrios de Luna) | 3004.79 mm (Vega de Urriellu, mean 2008-2014). |
| Dominant species (mean cover in percentage and standard deviation from 80 inventory plots at each community) | *Luzula caespitosa* (25.3 ± 20.2), *Festuca summilusitana* (23.3 ± 21.5), *Minuartia recurva* (7.78 ± 8.35) and *Thymus praecox* (6.8 ± 5.35). | *Carex sempervirens* (10.9 ± 10.4), *Silene acaulis (6.46* ± 9.87), *Helianthemum urrielense* (6.29 ± 6.81) and *Helianthemum canum* (5.09 ± 5.01). |

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

|  |  |  |
| --- | --- | --- |
|  | 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 (from 31/07/2021 to 19/09/2022). | Higher total germination means high potential of regeneration by seeds within the year after dispersal. |
| Autumn germination | Germination at mid-November (from 31/07/21 to 12/11/21. | Germination without cold stratification requirement. Seeds germinate fast and grow before winter. |
| Spring germination (relative) | Germination at mid-June relative to end of autumn (from 13/11/21 to 16/06/22. | Germination 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 delayed until high summer. |
| Germination  in cold conditions | Germination during cold period (from T mean ≤ 3 ºC until T mean > 3 ºC). | Germination of species able to germinate under snow-like conditions (zero degrees and darkness). Maximizes ability of taking advantage of water available during. |
| t50 | Time to reach 50% germination. Species under 50% germination were excluded from analysis (n = 72 seed lots). | Precocious vs. delayed germination along the year. Higher values indicate that germination tends to occur later in the year. |
| Environmental heat sum (EHS) | Sum of degrees (T mean) needed to reach t50. Species under 50% germination were excluded from analysis (n = 72 seed lots). | Physiological threshold to accumulate heat before germination. A species and individual trait, it should be independent of the incubator. |

Imagen que contiene exterior, foto, firmar, diferente

Descripción generada automáticamente 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.

Gráfico

Descripción generada automáticamente

Fig 2. Continuous seasonal temperature experiments performed at weekly resolution with daily temperature ramps and monthly photoperiods to represent fellfield and snowbed conditions in laboratory incubators. The experimental settings were based on soil temperatures measured during 10 years in alpine communities of the Picos de Europa National Park, Cantabrian Mountains, Spain. 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.

Gráfico, Gráfico de barras, Gráfico de cajas y bigotes

Descripción generada automáticamente

Fig 3. Seed germination responses to incubator chambers representing fellfield and snowbed microclimatic scenarios in species from Mediterranean and Temperate alpine communities of northern Spain. 3A. Effects of incubator according to the MCMC-glmm analysis of the data. Dots indicate mean effect size and whiskers are 95 % credible intervals (CI). The vertical dashed line marks the zero effect: when the CI crosses the zero-line, the effect is not significant; positive and significant effect sizes mean higher values in the snowbed incubator, while negative and significant effect sizes mean higher values in the fellfield incubator. 3B. Mean value for the eight traits at each incubator for both communities. Error bars in Total, Autumn, Spring, Summer, Winter conditions are binomial confident intervals. Error bars in t50 and EHS are standard error measures.

Gráfico, Gráfico de líneas

Descripción generada automáticamente

Fig 4. Cumulative germination curves, based on the sum of all species germination from each community (Mediterranean left panel (n=21) and Temperate right panel), throughout the experiment. Within each panel, orange curve represents germination proportion in fellfield incubator and blue curve snowbed incubator. Flat areas represent cold period when no germination scores were done.