# Microclimate regulates seed germination phenology in Temperate and Mediterranean alpine communities.

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## Abstract (max 250 words)

Understanding the plasticity of seed germination phenology 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 phenology. Mirroring yearly temperature data series recorded in alpine soils, we conducted a continuous seasonal temperature experiment with fresh seeds to investigate germination phenology in two contrasting microclimatic scenarios: fellfield (warmer temperatures and snow-free) 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 seven traits related to germination phenology, 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 the Mediterranean rather than in the temperate community, which experienced higher germination after cold stratification and warm temperatures. 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 alpine biome is characterized by extreme climatic conditions, short growing seasons, and high topographic roughness (Körner 2021). At fine scales, topographic roughness translates into a mosaic of microclimatic conditions (Scherrer, D. and Körner, 2011) especially incident in the snow-melting gradient and the temperature regimes (Körner 1999). Microclimatic conditions likely modify plant community assembly processes (Körner 2003) favoring the development of specific adaptations (Graham et al 2012). The micro-topographic gradient also determines local richness (Schöb 2009), compositional (Jiménez-Alfaro et al., 2014) and functional diversity (García-Gutiérrez et al., 2018). Thus, seems logical to think that the regeneration niche (Grubb 1977) may also differ according to this micro-topographic gradient. Recent studies highlight the importance of integrating regeneration traits in plant community ecology (Jiménez-Alfaro et al. 2016) and can explain an independent axis of variation (Hoyle et al. 2015). Germination, a commonly studied process within regeneration, is a sensitive (Hoyle et al. 2015) and irreversible process (REF). Therefore, germination strategies and phenology have strong fitness implications in plant development and survival (Donohue 2005). Recent studies defined a global “alpine germination syndrome”, characterized by the requirement of a period of cold stratification followed by warm temperatures to initiate germination (Baskin and Baskin 2014, Fernández-Pascual 2021). This strategy avoids adverse periods and ensure germination at the beginning of growing season when water is available, and seedlings have time to develop a root system before next winter (REF).

Even though this alpine syndrome has been detected, the data available behind it mostly originated in temperate regions from the northern hemisphere (REF), thus is geographically limited. Considering that germination strategies are highly variable in alpine ecosystems (Wagner and Simons 2009) it is reasonable to think that it might differ across latitudinal ranges. One example has been studied in the Mediterranean climate, a different germination syndrome was proposed for oromediterraneous regions with an alpine belt where species germinate immediately after dispersal if water is available and show improved germination after a period of cold stratification, referred to as “Mediterranean germination syndrome” (Jiménez-Benavides 2005). Nevertheless, we don’t fully understand the underlying causes of the variation observed; in case-specific studies, germination variability was explained by species altitudinal range (Fernández-Pascual et al, 2016), bedrock (Tudela-Isanta et al., 2017), species order of appearance in glacier foreland succession (Schwienbacher et al 2012) or species realized niche within the snowmelt gradient (Rosbakh et al. 2022). Thus, the mosaic of microclimatic conditions can be another potential source of variation. The few studies that consider microclimatic variability found no differing results (Shimono and Kudo 2005, Sergey??). Nonetheless, these studies measured germination based on standard laboratory procedures with seeds subjected to constant temperature treatments and/or focusing on a short period of time, generally four to six weeks. These experimental designs barely mimic real field climatic conditions and fluctuations, especially in the alpine environment (see “paper Picos”). To draw more robust conclusions about real-life germination strategies happening in alpine areas we need habitat specific detailed climatic data (Shimono and Kudo 2005) and accurate experimental settings that can provide that level of detail. Filling these knowledge gaps will help us understand and/or predict community responses to future climate scenarios.

The approach we present here is to study germination phenology across a whole year in two contrasting real microclimatic scenarios through a variety of germination traits. For the study purpose we defined two microclimatic scenarios: micro-edges (referred to as “fellfields”) i.e. more open and exposed areas subjected to freeze and thaw cycles without snow protections in winter and dry periods in summer; and micro-valleys (referred to as “snowbeds”) i.e. areas with dense plant cover, long snow cover and high moisture. The specific questions we want to investigate are: (1) will microclimatic scenarios 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 hypothesize that microclimatic scenarios 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. Thus, we expect fellfield scenario to result in faster germination, higher germination in early season, higher total germination and lower or no germination in winter. In snowbeds, we expect the opposite germination pattern. Considering our second question, we hypothesize 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. We established two study communities in two contrasting ecosystems of the Cantabrian high mountains (Fi g 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). Communities are dominated mostly by *Poaceae* and *Cyperaceae*, but they are also rich in Hemicryptophytes and Chamaephytes. The local alpine diversity is regulated by meso-topographic gradients that modify the relative abundance of species with Eurosiberian, Mediterranean or endemic origin (Jiménez-Alfaro et al., 2014). The temperate community presents higher richness with some relict alpine species; located above calcareous bedrock (pH 6.5 - 6.8, own non-published data) with average annual precipitation around 3000 mm endures less than 1-month dry periods (usually in summer). Growing season starts in May until early October with a mean annual temperature in the soil of 5ºC. Grazing impact is restricted to wild populations of Cantabrian chamois (*Rupricapra pyrenaica parva*). The Mediterranean community located above an acidic bedrock (pH 3.8 – 4.8, own non-published data) has a lower richness and experience 2-month dry period in summer (average annual precipitation is 1050 mm, mostly accumulated in spring and autumn). Growing season stretches from March to October with a mean annual temperature of 8ºC. Grazing impact is also restricted to wild populations of Cantabrian chamois.

### 2.1 Seed sampling

Within each community we established four seed sampling sites, separated at least 500 m from each other, assuming they form the same meta-population. In each collection site, we recorded all vascular plant species co-occurring in a central representative plot of 3 m radius. We considered the species recorded in the eight sites 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 collection sites every two weeks during the reproductive season and sampled ripe seeds or fruits (hereafter ‘seeds’ for simplicity) directly from the mother plants according to target species maturity peak. In general, we followed standard protocols for sampling seeds of wild populations to maximize 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 collection site, and we collected seeds from at least 20 - 50 individuals chosen at random. The goal was to collect at least 200 seeds from each target species in two sampling sites, maximizing spatial sampling within the community. In the temperate community we obtained seeds from 38 target species, of which 19 were represented by 2 sites. In the Mediterranean community we obtained enough seeds from 21 target species, covering two collection sites for each species except in 2 cases. Only three species (*Jurinea humilis*, *Thymus praecox* and *Silene ciliata*) were present in both communities (with two collection 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 microclimatic scenarios in snow cover period and temperatures to recreate those same conditions in incubation chambers. The final experimental scenarios consisted of a weekly-resolution temperature program (fig 2) with daily temperature ramps between daily Tmax and daily Tmin, and monthly-resolution photoperiod regimes. Each experimental scenario 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 continuously from July 2021 to September 2022. We will refer to the experimental chamber with warmer temperatures and snow-free mimicking micro-edge topography as “fellfield incubator” and the chamber with cooler temperatures and long snow period program mimicking micro-valleys will as “snowbed incubator”.

The major differences between incubators were the temperature regimes and the length and climatic conditions during the winter (defined as the period with T min < 1 ºC). In the fellfield incubator we programmed winter period with below 0 ºC temperatures with daily temperature and photoperiod fluctuation for 144 days while in the snowbed incubator we programmed constant 0 ºC and darkness for 196 days (see in Fig 2C). Consequently, the growing season after the winter also differed, with 176 days in the fellfield incubator and 115 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. Seed lots remained in room conditions between collection and sowing. We did a sequential sowing of each species according to their maturity peaks in the field, seeds entered the incubators at their optimum dispersal time. Each seed lot (species x collection site, N = 95) 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 winter, incubators remained closed to avoid increases of temperature that could trigger seed germination and therefore no water was added during that period.

We checked germination every two weeks before winter, and weekly after winter until the end of the experiment. As incubators remained closed during winter we did not perform germination scores in that period. We removed seeds from the petri dishes once germination occurred (radicle > 1.5 mm long). We cut open under the binocular loupe non-germinated seeds at the end of the experiment to visually assess if they were empty, infected, or looked normal. We considered seeds with white and firm embryos viable, i.e. potentially germinable (Baskin & Baskin, 2014). We did not consider empty or infected seeds 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 = 14), 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 95 seed lots of 54 species representing 21 plant families. A total of 10,816 viable seeds were used in the experiment.

### 2.4 Data analysis

We defined a set of traits to describe germination phenology (see Table 1). These traits were chosen to synthesize the diverse patterns observed during the continuous germination experiment, by considering their functional ecological significance in the alpine ecosystem. Autumn and summer germination consider specific periods of time measured in calendar dates (i.e., equal length between incubators). Winter germination is defined by a specific thermal threshold, from last germination score before winter (11th of November, T mean < 3.5 ºC) until T min > 1 ºC (i.e., different length between incubators). Spring germination consider germination from Tmin = 2 until summer solstice (germination score the 24th of June) also different lengths between incubators (see Fig 2C). Total germination considers the accumulated germination at the end of the experiment. 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 (N=68).

We analyzed the 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 traits, we used binomial MCMCglmms (family = multinomial2) while for the t50 and EHS traits we scaled the values and used gaussian MCMCglmms (family = gaussian). To calculate t50 trait in days, we fitted a linear model between the two 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.

To answer the first question, we analyzed each community separately and modeled every germination trait as response variable and incubator as explanatory variable (fixed factor), while phylogeny and collection site (nested within species) were specified as random factors (germination metric ~ incubator). To answer the second question, we tested each germination trait as response variable and both incubator and community as explanatory variables (fixed factors) paying special attention to their interaction while random factors remained the same (germination metric ~ incubator \* community). 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. 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).

## 3. Results

### 3.1. Microclimatic scenarios responses

Visualization of the cumulative germination data showed consistent differences between microclimatic scenarios in both communities (Fig 3).

In the fellfield scenario we observed significantly higher autumn and spring germination while the opposite results were obtained in summer and winter germination with higher values in snowbed scenario (see fig 4A for size effects and fig 4B for mean values). Total germination was slightly higher in fellfield scenario in both communities, but differences were only significant in the Temperate community. T50 values were consistently higher in snowbed scenario and EHS did not statistically differ between scenarios (see fig 4). The effect size was notably larger in the temperate community meaning that the differences observed between incubators were bigger.

### 3.2 Community responses

When analyzing community patterns, we found somewhat converging patterns but significantly different strategies between communities (see fig 3).

The temperate community showed low germination in autumn i.e., before cold stratification period (fig4B, left panel first row). In winter, we found low germination almost all happening in the snowbed scenario (fig 4b, left panel second row). In spring germination, we observed the highest germination in fellfield scenario (0.38 germination proportion) while the highest germination in snowbed scenario was reach during summer (0.31 germination proportion). At the end of the experiment total germination averaged 0.67 of germination proportion. T50 values averaged 203 days (163 and 242 in fellfield and snowbed respectively), and the average EHS was 470 ºC, very similar values independently of scenario.

The mediterranean community showed the highest germination proportion in autumn (0.58), followed by spring germination (0.15 on average). Low germination happened during winter, mainly in the snowbed scenario (0.12) and the lowest germination was recorded in summer with a mean value of 0.05. At the end of the experiment the Mediterranean community averaged 0.84 of germination proportion. T50 values averaged 84 days (64 and 102 in fellfield and snowbed scenarios respectively) and the average EHS was 289 ºC.

As expected, autumn germination was significantly higher in the Mediterranean rather than in temperate community (Appendix table xx). Germination in winter showed differences between microclimatic scenarios (same pattern in both communities), values more than 30 times higher in snowbed scenario. Spring germination was significantly higher in the temperate community. Summer germination was higher in snowbed scenarios for both communities with the highest values showed in temperate community. Total germination was significantly lower in the temperate community, although in both cases it was slightly higher in fellfield incubator. T50 values were significantly higher in the temperate community. The average t50 time was significantly higher in the temperate community. EHS was only significantly lower for temperate community in the snowbed scenario (see detailed results from community model in appendix table xx). We found a significant interaction term in all germination traits except total germination, being the temperate community in the snowbed scenario the most distinct one, with either higher or lower values.

4. Discussion.

As we predicted, the microclimatic scenarios drove significant differences in the germination phenology of our two alpine communities. Fellfield conditions (mirroring micro-edges) did prompt faster germination (t50), higher germination in early season (autumn + spring), higher total germination, and lower germination in winter conditions. Conversely, in snowbed conditions (mirroring micro-valleys) we observed slower germination, higher germination later in season, lower total germination, and higher germination in winter conditions. As we also expected, the temperate community diverged significantly from the Mediterranean community pattern. The temperate community followed the “alpine germination syndrome” (Baskin and Baskin 2014, Fernandez pascual 2021) with low germination before cold stratification and higher germination once the incubators reach warm temperatures (corresponding to spring germination in fellfield incubator and summer germination in snowbed incubator). Whereas the Mediterranean community showed the highest germination in autumn and earlier in the growing season (spring germination), when temperatures are still low, following the previously described “Mediterranean germination syndrome” (Gimenez Benavides 2005).

Párrafo 2 sobre las diferencias entre microclimas. Explicar la significación ecológica de las diferencias que hemos visto entre fellfield y snowbed, y relacionarlas con la literatura existente.

Our results confirm that microclimatic conditions can be one of the reasons of the high variability observed in alpine germination. Small microclimatic differences of two or three degrees resulted in high accumulated germination differences throughout a year. The differences generate a significant delay that potentially have a detrimental effect on plant demography since fecundity fitness and seedling survival are tightly related to the start and length of the growing season.

The higher autumn germination found in fellfield scenario, probably facilitated by the higher temperatures experienced has been also categorized as opportunistic by Hoyle 2015. It can provide selective advantage if seeds achieve ripening and shedding “early in the season” consequently, 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.

Germination in winter conditions showed consistent differences between microclimatic scenarios, suggesting that in both communities, the species are better adapted to germinate under snow-like conditions (snowbed scenario) than at below zero temperatures with light (fellfield scenario). These results suggest that snow-like conditions can alleviate and break dormancy while keep water available. On the contrary below 0ºC temperatures showed detrimental effects partly because of the physiological drought they are subjected to. Seeds that arrive to micro-valleys (snowbed) can start germination under snow are taking advantage of water availability during snowmelt using the full extent of their short growing season to develop seedlings leaves and roots. Seedling establishment is severely restricted by the ability to survive topsoil desiccation during summer and freezing events the following winter (REF). According to our results, seeds that disperse to micro-edges (fellfields) must be able to endure below 0 ºC temperatures and hold back germination until a rain event plus temperatures rise above at least 3 ºC according to our experiment. The coincidence of rain and temperature can potentially shorten their growing season depending on weather conditions. Nevertheless, according to our field data micro-edges reach that 3ºC at beginning of April therefore are likely able to develop until beginning of October.

The contrasting responses observed in the spring germination highlight the different germination syndromes in our communities. In the Temperate community only species that fulfilled the warm temperatures requirements were able to germinates, mostly in fellfield scenario. Nevertheless, in the Mediterranean communities we did not observed differences between scenarios, suggesting that species are better adapted to germinate at cooler temperatures.

In late growing season we observe a significant higher germination in snowbed scenario, where species finally reached their germination temperature requirement. In Mediterranean community most species where already germinated by then while in the temperate community showed the highest germination for the whole year.

Our results suggest that heat waves (events of higher temperatures) in the future could potentially advance germination phenology to earlier in the season leaving species vulnerable either to winter freezing (if they germinate in late autumn) o early spring freezing episodes (if they germinate in early spring) and are not protected by snow. Potential negative effect will be more apparent in the temperate communities because seedling might have evolved to develop in longer periods of time. 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 phenology (Mondoni 2012). In our experimental setting fellfield incubator (warmer) reproduced the same behaviour with faster germination rate and more autumn /spring.

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 phenology and strategy of it is what in the end will determine if will be or not successful.

T50 values exemplify the different rates experienced in both scenarios, with faster rates in fellfield scenario with warmer temperatures, also supporting the hypothesis that warmer temperatures in the future might accelerate germination.

The EHS lack of differences exemplifies that species requirements have a stronger influence on germination than in microclimatic scenarios, at least at community level.

MED: The cumulative germination curve showed that there is a significant germination delay between contrasting microclimatic conditions after the cold period. TEMP: The cumulative germination curve showed that there is a significant germination delay between microclimatic conditions from the beginning of the experiment.

Párrafo 3: lo mismo que el 2, pero sobre las diferencias del Mediterráneo sobre el templado.

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). Nevertheless, both communities reached elevated germination proportions, suggestions that both communities are successful in generating viable seeds and both strategies can be appropriate in alpine regions.

The temperate community showed a consistent trait differentiation in all germination traits except EHS. 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 Mediterranean community showed a consistent germination trait differentiation between microclimatic conditions except in total germination, spring germination and EHS. We found a higher proportion of non-dormant species (82%), able to germinate immediately after dispersal at both microclimatic conditions if water is available. Germination in spring (relative to end of autumn) did not differ between microclimatic conditions suggesting that species from the community are well adapted to germinate in early season. 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.

Párrafo 4: recalcar la novedad del estudio, basado en la calidad de los escenarios microclimáticos (resolución semanal, datos climáticos propios, etc.) y ADEMÁS repasando algunas limitaciones claras de nuestro estudio (comento 2 más arriba).

Our study focused on germination phenology 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.

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.

Microclimatic scenarios based in temperate community

2 annual species with same program the first august

Párrafo 5: direcciones futuras (óptimamente coincidiendo con el resto de trabajos que estás haciendo) y conclusiones finales.

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.

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)

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.

Intraspecific adaptations to microclimatic scenarios (species levels, next paper??)

Other alpine germination syndrome in tropic/arctic

Water availability

Seedling timing/stress/

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# Tables and figures

Table 2: Description of the seed traits describing germination phenology 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 |
| Autumn germination | Germination at mid-November, last check before winter (from 31/07/21 to 11/11/21). | Germination without cold stratification requirement. Seeds germinate fast and grow before winter. |
| Winter germination | Germination during cold period (from 12/11/21 until Tmin >1 ºC). Different length in each incubator. | Germination of species able to germinate under snow-like conditions (zero degrees and darkness). Maximizes ability of taking advantage of water available during. |
| Spring germination | Germination at the summer solstice relative to end of winter period (from Tmin > 1ºC to 24/06/22). Different length in each incubator. | Germination with cold stratification requirement but no need for high temperatures. Rapid onset of germination following snowmelt. |
| Summer germination | Germination at mid-September relative to spring (from 25/06/22 to 19/09/22). | Germination of species with cold stratification and warm cued germination. Germination delayed until high summer. |
| 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. Low values could potentially indicate a higher bet for long-term soil seed bank. |
| t50 | Time to reach 50% germination. Species under 50% germination were excluded from analysis (n = 68 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 = 68 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.

Diagrama

Descripción generada automáticamente

Fig 2A. Weekly means of soil temperatures (Tmax and Tmin at 5 cm depth) measured during 10 years in two alpine communities of the Picos de Europa National Park, Cantabrian Mountains, Spain. Fig 2B. Experimental temperature programs with weekly resolution, daily temperature ramps and monthly photoperiods to represent fellfield and snowbed scenarios in laboratory incubators. Both incubators were configured based on real field data showed in fig 2A. Fig 2C. Germination phenology periods considered in our study adapted to each microclimatic scenario.

Imagen que contiene Gráfico de líneas

Descripción generada automáticamente

Fig 3. Cumulative germination curves, based on all target species sowed from each community throughout the experiment. Within each panel, orange curve represents germination proportion in fellfield scenario and blue curve snowbed scenario. Flat areas represent cold period when no germination scores were done.

Gráfico, Gráfico de barras, Gráfico de rectángulos

Descripción generada automáticamente

Fig 4. Phenology traits responses to incubator chambers representing fellfield and snowbed microclimatic scenarios from Mediterranean and Temperate alpine communities of northern Spain. 4A. 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. Dots on the right side of the zero-line (positive) mean higher values in the snowbed scenario (blue background), while dots on the left side of the zero-line (negative) mean higher values in the fellfield scenario (orange background). 4B. Mean germination values for the seven traits at each scenario for both communities. Error bars in germination phenology traits autumn, winter, spring, summer and total are binomial confident intervals. Error bars in T50 and EHS are gaussian confident intervals.