# Phenological germination shifts of alpine plants in contrasting microclimates.

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## Abstract (max 250 words)

Understanding seed germination phenology is important to predict the resilience of plant communities to climate change; yet we know very little about the influence of microclimatic conditions on germination timing. Here, we conducted a continuous seasonal experiment with fresh seeds to investigate the germination phenology of species co-occurring in two alpine systems (acidic Mediterranean, calcareous temperate) using microclimatic long-term data series collected in alpine soils of northern Spain. The experiments were conducted for 57 species in two contrasting microclimatic scenarios based on (1) fellfield snow-free conditions with warmer temperatures, and (2) snowbed conditions with cooler temperatures. Using seven traits related to germination phenology, we found a consistent temporal delay in snowbed with respect to fellfield conditions in the two study systems, with stronger effects on the temperate system. A major axis of trait variation revealed species-based differences in autumn and spring germination, independently of the system or the microclimatic conditions. Despite macroecological effects resulting in lower dormancy and higher autumn germination in the Mediterranean than in the temperate system, we found the same species trait responses in the two microclimatic scenarios. These results suggest a generalizable phenological response of alpine plants to germinate in microclimatic conditions regulated by snow accumulation along mesotopographical gradients. In a warming scenario with reduced snow cover and higher temperatures, alpine species are expected to anticipate germination in different macroclimates, with disrupting effects on communities dominated by cold-adapted species.

## 1. Introduction

Recent studies highlight the importance of integrating regeneration traits in plant community ecology (Jiménez-Alfaro et al. 2016) to understand an independent axis of variation (Hoyle et al. 2015). As one of the key stages of plant regeneration, germination is a sensitive and irreversible process (Baskin & Baskin 2014). START HERE (what is phenology?)… Plant reproductive strategies have strong fitness implications for development and survival (Donohue 2005; Poschlod et al. 2013). Most studies have focused on flowering time, seed maturation and dispersal onset, describing a fast-slow continuum of reproductive phenology (Segrestin 2018, Segrestin 2019). Early season germination can benefit individuals with longer growing season (Donohue et al. 2010) and give a competitive edge to limiting resources (Verdú & Traveset 2005) against individuals germinating later during the season. However, early germination also involves higher mortality risks (Thomson et al. 2017) due to warm spells or frost event (REF) compared to a more conservative strategy i.e. seeds germinating later in the season (ten Brink 2020). Thus, even though germination timing proved to be influential for population and community dynamics in response to changes in climatic conditions (Kimball et al., 2011; Levine et al., 2011; Huang et al., 2016) little is known about seasonal germination phenology in harsh or unpredictable environments.

In alpine habitats, germination phenology is of vital importance to match favorable conditions and to prevent unsuitable winter climate (Gremer 2020). Consequently, germination traits have evolved following diverse strategies; adopting a bet-hedging strategy, i.e. spreading the mortality risk with several germination episodes (Simons, 2011), or having specific environmental germination cues that trigger the germination process when abiotic conditions are favorable (i.e. temperature, moisture or light) (Donohue 2010, Baskin and Baskin 2014) or a combination of both (Simons 2014). The global alpine germination syndrome has been characterized by the requirement of a period of cold stratification followed by warm temperatures to initiate germination (Baskin & Baskin 2014; Fernández-Pascual et al. 2021). This strategy avoids adverse periods and ensure germination at the beginning of growing season when water is available, thus seedlings have time to develop a deep root system before summer drought periods and the following winter (Billings & Mooney 1968). Even though this alpine syndrome has been detected, the data available behind it mostly originated in temperate regions (Fernández-Pascual et al. 2021), thus is geographically limited. Considering that germination strategies are highly variable in alpine ecosystems (Wagner & Simons 2009) it is reasonable to think that they might differ across latitudinal ranges. For example, a different germination syndrome was proposed for high altitude areas with Mediterranean climate where species germinate immediately after dispersal if water is available and show improved germination after cold stratification, referred to as “Mediterranean germination syndrome” (Giménez-Benavides et al. 2005). Nevertheless, the underlying causes of the variation observed deviating from the main germination syndromes are still unknown. In case-specific studies, germination variability was partly explained by species altitudinal range (Fernández-Pascual et al., 2017), bedrock (Tudela-Isanta, Fernández-Pascual, et al. 2018; Tudela-Isanta, Ladouceur, et al. 2018), species order of appearance in glacier foreland succession (Schwienbacher et al. 2012) or species realized niche within the snowmelt gradient (Rosbakh et al. 2022).

The alpine habitat is characterized by extreme and changing climatic conditions, short growing seasons, and high topographic roughness (Körner 2021). At fine scales, topographic roughness translates into a mosaic of microclimatic conditions (Scherrer & Körner 2011) especially incident in the snow-melting gradient and the temperature regimes (Körner 2021) showing sharp gradients over a few centimeters (Graham et al. 2012) and likely modifying plant community assembly processes and favoring the development of specific adaptations (Körner 2021). The micro-topographic gradient also determines local richness (Schöb et al. 2009), compositional (Jiménez-Alfaro et al. 2014), and functional diversity (García-Gutiérrez et al. 2018). Thus, it seems logical to think that the regeneration niche (Grubb 1977) may also differ according to microclimatic conditions along topographical gradients. However, the few studies that consider microclimatic variability found contrasting results. (Shimono & Kudo 2005) found no differences between fellfield and snowbeds specialist while Rosbakh et al. (2022) did found that species germination was affected by the position along the snowmelt gradient. Nonetheless, these studies are based on standard laboratory procedures with seeds subjected to specific constant treatments throughout the experiment and for a short period of time. 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 & Kudo 2005) and accurate experimental settings that can provide that level of detail. Filling these knowledge gaps will help us understand and predict community responses to future climate scenarios.

The approach we present here is to study germination phenology across a whole year in two contrasting microclimatic scenarios through a variety of germination traits. For the study purpose, we defined two microclimatic scenarios: one mimicking exposed micro-edges (referred to as “fellfield scenario”) i.e., more open and exposed areas subjected to wind, freeze and thaw cycles without snow protections in winter, and warmer summers; and the other mimicking micro-valleys (referred to as “snowbed scenario”) i.e., areas with dense plant cover, long snow cover and cool summers. 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 our first question, we hypothesize that microclimatic scenarios will result in differences in the germination phenology of alpine communities. Exposed micro-edges have higher mean annual temperatures, longer growing seasons, shorter winters, and more frequent freezing events than micro-valleys. Thus, we expect the fellfield scenario to result in higher total germination, higher germination in early season, and lower or no germination during winter. In the snowbed scenario, we expect the opposite germination pattern with lower total germination, higher germination in late season and some germination happening under winter conditions. Considering our second question, we hypothesize that a temperate alpine system will show a higher concordance with the global alpine germination syndrome (Fernández-Pascual et al. 2021) than a Mediterranean alpine system. In the temperate alpine system, we expect more germination after cold stratification and under warmer temperatures, while in the Mediterranean system we expect faster germination and germination at colder temperatures (Giménez-Benavides et al. 2005).

## 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 (Fig 1A). 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 1A). 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 systems in two contrasting ecosystems of the Cantabrian high mountains: (1) Mediterranean high mountain acidic grasslands in the Valles de Omaña and Luna Biosphere Reserve (SW of the Cantabrian mountains, fig 1A in red); and (2) temperate alpine calcareous grasslands in the Picos de Europa National Park (NE of the Cantanbrian mountains, fig 1A in blue). Alpine grasslands communities in the area are dominated mostly by *Poaceae* and *Cyperaceae*, rich in Hemicryptophytes and Chamaephytes. The temperate system presents higher richness (n= XX) with some relict alpine species. 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). This system is located above calcareous bedrock (pH 6.5 - 6.8, own data), mean annual air temperature is 2.5 ºC while mean summer precipitation is 260 kg·m2 (based on 47 locations, values extracted from Chelsa 2.1, bio 1 and bio 17, fig 1B). Growing season starts in May until early October with a mean annual soil temperature of 5ºC. The Mediterranean system, with lower plant richness (n= 3-14), is located above an acidic bedrock (pH 3.8 – 4.8, own data), it experiences a 2-month dry period in summer (average precipitation of 160 kg·m2) and mean annual air temperature is 4.5 ºC (fig 1B). Growing season stretches from March to early November with a mean annual soil temperature of 8ºC. Grazing impact is restricted to wild populations of Cantabrian chamois (*Rupricapra pyrenaica parva*) in both systems.

## 2.2 Continuous germination experiment with microclimatic scenarios

We performed a continuous seasonal temperature experiment to mimic one year of temperature regimes based on field soil temperatures previously recorded in the region. 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 micro-valleys (snowbeds) and micro-edges (fellfields). 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, fig 2A) 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 sites as climatic scenarios in snow cover period and temperatures to recreate those same conditions in incubation chambers (fig 2A). The final experimental scenarios consisted of a weekly-resolution temperature program (fig 2B) 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 incubator with warmer temperatures and snow-free mimicking micro-edge topography as “fellfield scenario” and the incubator with cooler temperatures and long snow period program mimicking micro-valleys will as “snowbed scenario”.

The major differences between incubators were the temperature regimes and the length and climatic conditions during the winter. In the fellfield scenario we programmed winter period with below 0 ºC temperatures, and daily temperature and photoperiod fluctuation for 144 days. While in the snowbed scenario we programmed constant 0 ºC and darkness for 196 days. Consequently, the growing season length also differed, with 176 days in the fellfield scenario and 115 days in the snowbed scenario. Mean temperature differences between our two scenarios during the growing season were around three degrees Celsius each week (more detailed information about weekly programs in Supplementary table Xx) reaching a maximum mean temperature of 18.5 ºC in the fellfield scenario and 15.5 ºC in the snowbed scenario, both during last week of August.

## 2.3 Seed sampling and germination trails

Within each system 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 collection sites as the target community in our experiment (49 species in the temperate and 22 species in the Mediterranean systems).

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 the field. In general, we followed standard protocols for sampling seeds of wild populations to maximize intraspecific genetic diversity (ENSCONET 2009). 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 system we obtained seeds from 38 target species, of which 19 were represented by 2 sites. In the Mediterranean system 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 systems (with two collection sites sampled in each system).

Fresh seeds were manually cleaned and sown within 25 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, meaning 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. Incubators remained closed during winter thus, 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 (raw data in Supplementary 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 Germination traits

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 seasonal experiment, by considering their functional ecological significance in the alpine ecosystem. The first four consider the phenological season: 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 (T mean < 3.5 ºC) until T mean > 2 ºC (i.e., different length between incubators). Finally, spring germination consider germination from T mean > 2 ºC until summer solstice (germination score the 24th of June), having also different lengths between incubators (Fig 2A germination phenology periods). We also calculated two traits related to germination speed: 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). Finally, we considered total germination calculated as the accumulated germination by the end of the experiment.

## 2.5 Statistical analysis

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, will microclimatic scenarios modify the germination phenology of alpine communities? We analyzed each system separately and modeled (with MCMCglmm) 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 trait~ incubator). To answer the second question, do alpine communities from different macroclimates show different patterns of germination phenology? We tested each germination trait as response variable and both incubator and system as explanatory variables (fixed factors) paying special attention to their interaction. Random factors remained the same as the first model (germination trait ~ incubator \* system). 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).

To further explore the relationship between species and germination traits we divided the raw data in 4 subsets, one per each system at each incubator (fig 4) and perform a PCA (FactoMineR package REF). To select non redundant traits we first did a correlation analysis (plot with corrplot package REF) of all seven traits (Supplementary fig XXX), considering the high correlation between t50 and autumn germination (-0.94) we decided to keep the later.

## 3. Results

## 3.1 Germination patterns and species variability

The cumulative germination curves (considering all species) in each system (fig 3A) show a noticeable delay between incubators. The delay is represented by the area between curves density plot; which is a measurement of the space between the both germination curves (one for each incubator) of every species (see individual species germination curves in Supplementary XX). In the temperate system the density plot is wider which mean that more species had larger areas between curves i.e. more different responses between incubators. In the Mediterranean density plot most values are close to 0, meaning that species had smaller differences in germination between incubators. The interspecific variation is shown in fig 3B and 3C, where individual species germination curves are presented for each incubator. In fellfield incubator (fig 3B), and especially in the Mediterranean system, we can observe a vast majority of the curves peaking before winter period (71%, 15/21 sp) while in the temperate system species germination peak is divided between autumn (31.6%, 12/38 sp) and spring period (44.7%, 17/38 sp). Meanwhile in snowbed incubator (fig 3C), most species of the temperate system peaked in summer period (36.8%, 14/38) on the contrary in the Mediterranean system most species still germinated in autumn period (57.1 %, 12/21 sp). Detailed individual species germination peak periods and delay to reach 50% germination can be checked at table 2 (Temperate species) and table 3 (Mediterranean species).

The temperate system showed a significant germination delay between microclimatic scenarios from the very beginning of the experiment (fig 3A). This system showed low germination in autumn, and low germination in winter, basically only happening in the snowbed scenario (fig 5B, left panel second row). In fellfield scenario, we observed the highest germination period in spring (0.38 germination proportion) while the highest germination period in snowbed scenario was reach during summer (0.31 germination proportion). At the end of the experiment, total germination was significantly higher in fellfield scenario. T50 values were higher in snowbed scenario with an average of 242 days, while EHS values were very similar independently of the microclimatic scenario (detailed individual species traits in supplementary table xxx).

The Mediterranean system showed a germination delay between microclimatic scenarios mainly after the winter period. This system showed the highest germination proportion in autumn (0.58), followed by spring germination (0.15). Little germination happened during winter, mainly in the snowbed scenario (0.12) and the lowest germination was recorded in summer. At the end of the experiment the Mediterranean system averaged 0.84 of germination proportion and no significant differences between scenarios. T50 values were significantly higher in snowbed incubator (102 days on average) and EHS values were very similar independently of the microclimatic scenario (detailed individual species traits in supplementary table xxx).

### 3.3. Microclimate responses (germination traits)

We observed the same response pattern to microclimate in all our traits between both systems. In the fellfield scenario we observed significantly higher autumn and spring germination while we obtained the opposite results in summer and winter germination with higher values in snowbed scenario (fig 5A: model size effects and fig 5B: mean values). Total germination was slightly higher in fellfield scenario in both systems, but differences were only significant in the temperate system. T50 values were consistently higher in snowbed scenario and EHS did not statistically differ between scenarios (fig 5A). The effect size was notably larger in the temperate system meaning that the differences observed between scenarios were bigger, also supporting the results observed in the density plot of area between curves (detailed germination trait values per species can be checked at Supplementary XX).

## 3.2 Micro-Macro Interaction

When comparing strategies between systems we found significant different dominant strategies. As expected, autumn germination was significantly higher in the Mediterranean rather than in temperate system (See fig 5B 1st row and supplementary table xx). Germination in winter and summer periods did not differ between systems in both cases with significantly higher values in snowbed scenario (fig 5B 2nd and 4th rows). Spring germination showed significant differences with higher values in the temperate system (see fig 5B 3rd row) while total germination was significantly lower in the temperate system. To conclude, the average T50 time was significantly higher in the temperate system and EHS was only significantly lower for temperate system in the snowbed scenario (see detailed results from system model in appendix table xx). We found a significant interaction term in all germination traits except total germination, being the temperate system in snowbed scenario showing the most distinct responses, with either higher or lower germination values. This result is corroborated by the exploratory PCA where also the temperate system in snowbed incubator show different driving traits.

## 3.4 Species variation

The exploratory PCAs (fig 4) showed that in fellfield scenario both systems (although composed by completely different species, only 3 common) are driven/modulated by the same traits. The first axis (explaining 40% of variation) is majorly explained by autumn germination (and spring in the opposite direction), the second axis (31% of variation) is mainly explained by summer germ and 3rd axis (17% of variation) by winter. Contrastingly, in the snowbed scenario systems responded differently. In the Mediterranean system the first axis (explaining 40% of variation) was mainly correlated to autumn germination, the second axis (26.7 % of variation) to winter germ and the third axis (18% of variation) mainly explained by summer germination. On the other hand, the Temperate system first axis (explaining 42% of variation) was mainly explained by summer germination and EHS. Second axis (26.3% of variation) was mainly related to autumn germination and 3rd axis (21.3%) to spring germination (see detailed values of traits contributions and axis eigenvalues explanation in Supplementary xxx).

## 4. Discussion.

As we predicted, the microclimatic scenarios drove significant differences in the germination phenology of our two alpine systems. Fellfield scenario (mirroring micro-edges) did prompt faster germination, higher germination in early growing season, slightly higher total germination, and lower germination during winter. Conversely, in snowbed scenario (mirroring micro-valleys) we observed slower germination, higher germination in late growing season, slightly lower total germination, and higher germination in winter. The differences observed confirm that microclimatic conditions are an important source of variation in alpine germination phenology at community level. As we also expected, the temperate system diverged significantly from the Mediterranean system pattern. The temperate system followed the “alpine germination syndrome” (Baskin & Baskin 2014; Fernández-Pascual et al. 2021) with low germination before cold stratification and higher germination once the incubators reach warm temperatures. Whereas the Mediterranean system showed the highest germination in autumn and earlier in the growing season, following the previously described “Mediterranean germination syndrome” (Giménez-Benavides et al. 2005).

## 4.1 Microclimatic shifts

Small microclimatic differences of two or three degrees Celsius resulted in high accumulated germination differences throughout the year. The differences are shown as a phenology delay that can 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 (Poschlod et al. 2013).

In communities living in micro-edges (fellfield scenario), we observed two germination peaks. The first peak in autumn, can be seen as an opportunistic strategy (Hoyle et al. 2015). This strategy would provide selective advantage because seedlings can initiate development before the next growing season (REF), but only if seedlings can survive winter conditions as seen by (Mondoni et al. 2015) in glacier forelands. However, ultimately the success surviving winter seems to be species-specific and more studies are needed. Below zero temperatures showed consistent detrimental effects on germination although cold stratification is provided, a possibly reason could be the physiological drought they are subjected to (Billings & Mooney 1968). Consequently, seeds that disperse into micro-edges must endure below 0 ºC temperatures and postpone germination until frozen soil thaw. This postponed germination could potentially shorten their effective growing season; nevertheless, this delay is compensated by the shorter winter period experienced. The second germination peak is triggered when temperatures rise again in early growing season and almost no germination happens in late growing season.

In communities living in micro-valleys germination peaks occurs either under snow or later in the season. Micro-valleys conditions reduced autumn germination, partly because the low temperatures experienced and seeds dormancy constraints. Contrastingly, the winter germination peak demonstrate that alpine communities are adapted to germinate under snow-like conditions (darkness and constant 0ºC). These conditions alleviate and break seed dormancy while providing water during snowmelt. Thus, species able to germinate in that very restricted conditions are using the full extent of their growing season. Additionally, species with warm temperature requirements are not fulfilled until late growing season when we observed the second germination peak.

## 4.2 Macroclimate differences

The temperate system followed the previously described alpine germination syndrome (Baskin & Baskin 2014; Fernández-Pascual et al. 2021) corroborating the findings by many authors (Cavieres & Arroyo 2000; Mondoni et al. 2009; Schwienbacher et al. 2011; Hoyle et al. 2015). Accordingly, we observed low autumn germination likely due to a strong prevalence of physiological dormancy (Schwienbacher et al. 2011; Tudela-Isanta, Ladouceur, et al. 2018). Then, having a postponed germination after winter guarantee a drought-free period during snowmelt; but the highest germination was observed once the temperatures rose above 10-12 ºC. In micro-ridges (fellfield scenario) the thermal threshold was surpassed earlier in the growing season while in micro-valleys (snowbed scenario) it was exceeded later in the growing season. The total germination registered in this system suggests that some species might have a bet-hedging strategy, probably with fluctuating levels of dormancy and more prone to form persistent soil seed bank (Schwienbacher et al. 2010).

The Mediterranean system followed the previously described Mediterranean germination syndrome (Giménez-Benavides et al. 2005). Accordingly, we found a higher proportion of non-dormant species (82%), able to germinate immediately after dispersal when water was available confirming Giménez-Benavides results. The second germination peak was observed in early growing season suggesting that species from the system are well adapted to germinate at cooler temperatures. The higher values of total germination reached in the Mediterranean system suggest that might be more successful in generating viable seeds germinating within the first year, but also meaning that soil seed bank might not be persistent. The significantly distinct patterns observed in our two systems potentially indicate the existence of other germination syndromes still unknown in distinct biomes like tropical or artic areas.

In addition, the differential responses observed from the temperate system in snowbed scenario suggests that there are species with stricter germination requirements that will be the most vulnerable to climate warming.

## 4.3 Novelty, limitations, and further research

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 conditions in two alpine systems from the same biogeographical area. Our two systems differed also in bedrock potentially having a cofounding factor in our study, as germination trait might differ between siliceous and calcareous bedrock in the Alps (Tudela-Isanta, Ladouceur, et al. 2018). Although the microclimatic scenarios were based in the temperate system field data; the Mediterranean system showed high germination responses suggesting that these scenarios did not limit the germination capacity of the community and are therefore valid for our study purposes.

Another point worth considering is that in our experimental design seeds had water available throughout the growing season. Although literature agrees that temperature is the main factor influencing germination (Baskin & Baskin 2014; Körner 2021); the results of the Mediterranean system in our experiment, with species able to germinate at 5ºC, suggests that water availability may potentially have a stronger influence in germination than temperature in specific areas (Rosbakh & Poschlod 2015). Some studies that have approached the relationship between temperature and water availability in the soil showed that drier soils also become warmer, however to our knowledge none has been done at a microscale level (Graham 2012). Another study (Dürr et al. 2015) found that species inhabiting warmer soils tends to have a higher base temperature for germination, as they have adapted to need more heat as a cue to initiate germination. This adaptation could possibly be to prevent germination at dispersal time (Tudela-Isanta, Fernández-Pascual, et al. 2018). Nevertheless, there is still many gaps as how water availability directly affects germination, especially in water-limited ecosystems.

Looking at the generally high levels of germination reached in our study another important point would be to focus on seedling establishment and survival. Especially in alpine communities, seedlings phases have been identified as one of the more vulnerable and limiting stages of plant regeneration (REF). Seedling emergence must be perfectly timed to be successful. Germination before winter or in early spring can lead to mortality due to frost events (Rosbakh & Poschlod 2015; Fernández-Pascual et al. 2017); but with postponed germination the seedlings are at risk to an underdeveloped root system in summer period, when drought events can lead to death due to topsoil desiccation (Billings & Mooney 1968).

## 4.4 Conclusions

In future climate scenarios, with warming happening faster above the tree line (Kullman 2004 and Körner 2023), our results suggest that germination phenology could potentially advance to earlier in the season if individual species thermal requirements are met, confirming results found by (Mondoni et al. 2015; Bernareggi et al. 2016). Nevertheless, a greater threat might come from the expanding micro-edge climatic conditions, consequently reducing the suitable area of the specialized communities adapted to micro-valleys, specially from temperate alpine areas. The seemingly stricter germination requirements in temperate systems will no longer be met and germination phenology alterations could have major implications (Mondoni et al. 2012). Like reducing the amount of successful germination and leaving seedlings vulnerable to frozen damage without snow protection (Venn et al. 2013). Nevertheless, more research is needed to study species specific adaptations to microclimatic conditions, as well as their responses to limited water availability.

# 5. References

Baskin, C.C., & Baskin, J.M. 2014. *Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination*. Academic Press, San Diego, CA, USA.

Bernareggi, G., Carbognani, M., Mondoni, A., & Petraglia, A. 2016. Seed dormancy and germination changes of snowbed species under climate warming: The role of pre-And post-dispersal temperatures. *Annals of Botany* 118: 529–539.

Billings, W.D., & Mooney, H.A. 1968. The ecology of arctic and alpine plants. *Biological Reviews* 43: 481–529.

Cavieres, L.A., & Arroyo, M.T.K. 2000. Seed germination response to cold stratification period and thermal regime in Phacelia secunda (Hydrophyllaceae): Altitudinal variation in the mediterranean Andes of central Chile. *Plant Ecology* 149: 1–8.

Donohue, K. 2005. Seeds and seasons: interpreting germination timing in the field. *Seed Science Research* 15: 175–187.

Dürr, C., Dickie, J.B., Yang, X.Y., & Pritchard, H.W. 2015. Ranges of critical temperature and water potential values for the germination of species worlwide: contribution to a seed trait database. *Agricultural and Forest Meteorology* 200: 222–232.

ENSCONET. 2009. *ENSCONET Seed Collecting Manual for Wild Species*.

Fernández-Pascual, E., Carta, A., Mondoni, A., Cavieres, L.A., Rosbakh, S., Venn, S., Satyanti, A., Guja, L., Briceño, V.F., Vandelook, F., Mattana, E., Saatkamp, A., Bu, H., Sommerville, K., Poschlod, P., Liu, K., Nicotra, A., & Jiménez-Alfaro, B. 2021. The seed germination spectrum of alpine plants: a global meta-analysis. *New Phytologist* 229: 3573–3586.

Fernández-Pascual, E., Jiménez-Alfaro, B., & Bueno, A. 2017. Comparative seed germination traits in alpine and subalpine grasslands: higher elevations are associated with warmer germination temperatures. *Plant Biology* 19: 32–40.

García-Gutiérrez, T., Jiménez-Alfaro, B., Fernández-Pascual, E., & Müller, J. V. 2018. Functional diversity and ecological requirements of alpine vegetation types in a biogeographical transition zone. *Phytocoenologia* 48: 77–89.

Giménez-Benavides, L., Escudero, A., & Pérez-García, F. 2005. Seed germination of high mountain Mediterranean species: Altitudinal, interpopulation and interannual variability. *Ecological Research* 20: 433–444.

Graham, E., Rundel, P., Kaiser, W., Lam, Y., Stealey, M., & Yuen, E. 2012. Fine-scale patterns of soil and plant surface temperatures in an alpine fellfield habitat, white mountains, California. *Arctic, Antarctic, and Alpine Research* 44: 288–295.

Grubb, P.J. 1977. the Maintenance of Species-Richness in Plant Communities: the Importance of the Regeneration Niche. *Biological Reviews* 52: 107–145.

Hadfield, J.D. 2010. MCMCglmm: MCMC Methods for Multi-Response GLMMs in R. *Journal of Statistical Software* 33: 1–22.

Hoyle, G.L., Steadman, K.J., Good, R.B., McIntosh, E.J., Galea, L.M.E., & Nicotra, A.B. 2015. Seed germination strategies: An evolutionary trajectory independent of vegetative functional traits. *Frontiers in Plant Science* 6: 1–13.

Jiménez-Alfaro, B., Carlón, L., Fernández-Pascual, E., Acedo, C., Alfaro-Saiz, E., Redondo, R.A., Cires, E., del Egido Mazuelas, F., del Río, S., Díaz-González, T.E., García-González, M.E., Lence, C., Llamas, F., Nava, H., Penas, Á., Rodríguez Guitián, M.A., & Vázquez, V.M. 2021. Checklist of the vascular plants of the Cantabrian Mountains. *Mediterranean Botany* 42: 1–60.

Jiménez-Alfaro, B., Marcenó, C., Bueno, Á., Gavilán, R., & Obeso, J.R. 2014. Biogeographic deconstruction of alpine plant communities along altitudinal and topographic gradients. *Journal of Vegetation Science* 25: 160–171.

Jiménez-Alfaro, B., Silveira, F.A.O., Fidelis, A., Poschlod, P., & Commander, L.E. 2016. Seed germination traits can contribute better to plant community ecology. *Journal of Vegetation Science* 27: 637–645.

Jin, Y., & Qian, H. 2019. V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants. *Ecography* 42: 1353–1359.

Körner, C. 2021. *Alpine Plant Life* (Springer Nature Switzerland AG 2021, Ed.). Springer Cham.

M. Pagel. 1999. Inferring the historical patterns of biological evolution. *Nature* 401: 877–884.

Mondoni, A., Daws, M.I., Belotti, J., & Rossi, G. 2009. Germination requirements of the alpine endemic Silene elisabethae Jan: Effects of cold stratification, light and GA3. *Seed Science and Technology* 37: 79–87.

Mondoni, A., Pedrini, S., Bernareggi, G., Rossi, G., Abeli, T., Probert, R.J., Ghitti, M., Bonomi, C., & Orsenigo, S. 2015. Climate warming could increase recruitment success in glacier foreland plants. *Annals of Botany* 116: 907–916.

Mondoni, A., Rossi, G., Orsenigo, S., & Probert, R.J. 2012. Climate warming could shift the timing of seed germination in alpine plants. *Annals of Botany* 110: 155–164.

Poschlod, P., Abedi, M., Bartelheimer, M., Drobnik, J., Rosbakh, S., & Saatkamp, A. 2013. *Seed Ecology and Assembly Rules in Plant Communities*.

Rosbakh, S., Fernández-Pascual, E., Mondoni, A., & Onipchenko, V. 2022. Alpine plant communities differ in their seed germination requirements along a snowmelt gradient in the Caucasus. *Alpine Botany* 132: 223–232.

Rosbakh, S., & Poschlod, P. 2015. Initial temperature of seed germination as related to species occurrence along a temperature gradient. *Functional Ecology* 29: 5–14.

Scherrer, D., & Körner, C. 2011. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. *Journal of Biogeography* 38: 406–416.

Schöb, C., Kammer, P.M., Choler, P., & Veit, H. 2009. Small-scale plant species distribution in snowbeds and its sensitivity to climate change. *Plant Ecology* 200: 91–104.

Schwienbacher, E., Marcante, S., & Erschbamer, B. 2010. Alpine species seed longevity in the soil in relation to seed size and shape - A 5-year burial experiment in the Central Alps. *Flora: Morphology, Distribution, Functional Ecology of Plants* 205: 19–25.

Schwienbacher, E., Navarro-Cano, J.A., Neuner, G., & Erschbamer, B. 2012. Correspondence of seed traits with niche position in glacier foreland succession. *Plant Ecology* 213: 371–382.

Schwienbacher, E., Navarro-Cano, J.A., Neuner, G., & Erschbamer, B. 2011. Seed dormancy in alpine species. *Flora: Morphology, Distribution, Functional Ecology of Plants* 206: 845–856.

Shimono, Y., & Kudo, G. 2005. Comparisons of germination traits of alpine plants between fellfield and snowbed habitats. *Ecological Research* 20: 189–197.

Tudela-Isanta, M., Fernández-Pascual, E., Wijayasinghe, M., Orsenigo, S., Rossi, G., Pritchard, H.W., & Mondoni, A. 2018. Habitat-related seed germination traits in alpine habitats. *Ecology and Evolution* 8: 150–161.

Tudela-Isanta, M., Ladouceur, E., Wijayasinghe, M., Pritchard, H.W., & Mondoni, A. 2018. The seed germination niche limits the distribution of some plant species in calcareous or siliceous alpine bedrocks. *Alpine Botany* 128: 83–95.

Venn, S., Morgan, J.W., & Lord, J.M. 2013. Foliar freezing resistance of Australian alpine plants over the growing season. *Austral Ecology* 38: 152–161.

Wagner, I., & Simons, A.M. 2009. Divergence in Germination Traits among Arctic and Alpinepopulations of Koenigia islandica: Light Requirements. *Plant Ecology* 204: 145–153.

Zhang, Y., Chen, W., Smith, S.L., Riseborough, D.W., & Cihlar, J. 2005. Soil temperature in Canada during the twentieth century: Complex responses to atmospheric climate change. *Journal of Geophysical Research D: Atmospheres* 110: 1–15.

# Tables and figures

Table 1: Description of the germination phenology traits calculated in our study along with their ecological significance. Traits were calculated for petri dish and used as replicates per each seed lot.

|  |  |  |
| --- | --- | --- |
|  | Description | Ecological significance |
| Autumn germination | Germination at mid-November, last check before winter (T mean < 3.5 ºC). | Germination without cold stratification requirement. Seeds germinate fast and seedlings can develop before winter. |
| Winter germination | From last germination score before winter, T mean < 3.5 ºC, until T mean > 2 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 Tmean > 2ºC to summer solstice). 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. | Germination of species with cold stratification and warm cued germination. Germination delayed until late growing season. |
| Total germination | Total amount of seeds germinated at the end of the experiment. | 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. |

Table 2. Species list of the temperate system along the penology period with highest germination recorded at each incubator and the calculated area between species cumulative germination curves for each incubator (see individual species graphs in supplementary XX)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Family | Phenology period with highest germination | | Area between curves |
| Felfield | Snowbed |
| Androsace villosa | Primulaceae |  | Summer | -0.6 |
| Anthyllis vulneraria | Fabaceae | Autumn | Autumn | 12.2 |
| Arenaria erinacea | Caryophyllaceae | Autumn | Autumn | 69.1 |
| Arenaria moehringioides | Caryophyllaceae | Spring | Summer | 30.8 |
| Armeria cantabrica | Plumbaginaceae | Spring | Summer | 30.8 |
| Carex sempervirens | Cyperaceae | Spring | Summer | 38.2 |
| Dethawia splendens | Apiaceae | Spring | Winter | 11.9 |
| Erigeron alpinus | Asteraceae | Spring | Spring | 43.8 |
| Euphrasia salisburgensis | Orobanchaceae | Summer | Winter | 0.1 |
| Festuca hystrix | Poaceae | Autumn | Autumn | 83.2 |
| Festuca rubra | Poaceae | Spring |  |  |
| Gentiana verna | Gentianaceae | Spring | Winter | 1.2 |
| Gypsophila repens | Caryophyllaceae | Spring | Summer | 75.4 |
| Helianthemum canum | Cistaceae | Autumn | Autumn | 29.8 |
| Helianthemum urrielense | Cistaceae | Autumn | Autumn | -32.5 |
| Helictotrichon sedenense | Poaceae | Spring | Winter | 23.0 |
| Iberis carnosa | Brassicaceae | Autumn | Summer | 183.4 |
| Jasione cavanillesii | Campanulaceae | Spring | Summer | 14.0 |
| Jurinea humilis | Asteraceae | Spring | Winter | 94.1 |
| Koeleria vallesiana | Poaceae | Autumn | Autumn | 54.5 |
| Minuartia CF | Caryophyllaceae | Autumn | Summer | 97.2 |
| Minuartia verna | Caryophyllaceae | Autumn | Autumn | 112.4 |
| Pedicularis pyrenaica | Orobanchaceae | Spring | Winter | 21.1 |
| Phyteuma orbiculare | Campanulaceae | Spring | Winter | -11.4 |
| Plantago alpina | Plantaginaceae | Spring | Winter | 83.0 |
| Ranunculus carinthiacus | Ranunculaceae | Spring | - | 24.7 |
| Saxifraga conifera | Saxifragaceae | Autumn | Summer | 195.2 |
| Saxifraga paniculata | Saxifragaceae | Spring | Summer | 84.8 |
| Scilla verna | Asparagaceae | Summer | Summer | -1.4 |
| Sesleria caerula | Poaceae | Autumn | Summer | 146.8 |
| Silene acaulis | Caryophyllaceae | Summer | Summer | 17.5 |
| Silene ciliata | Caryophyllaceae | Spring | Spring | 56.6 |
| Thymus praecox | Lamiaceae | Autumn | Summer | 151.8 |

Table 3. Species list of the Mediterranean system along the penology period with highest germination recorded at each incubator and the calculated area between species cumulative germination curves for each incubator (see individual species graphs in supplementary XX)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Species | Family | Phenology period with highest germination | | Area between curves |
| Felfield | Snowbed |
| Agrostis tileni | Poaceae | Spring | Spring | 69.8 |
| Armeria duriaei | Plumbaginaceae | Autumn | Autumn | 25.4 |
| Cerastium ramosissimum | Caryophyllaceae | Autumn | Autumn | -8.0 |
| Conopodium majus | Apiaceae | Spring | Winter | 29.2 |
| Dianthus langeanus | Caryophyllaceae | Autumn | Autumn | 6.1 |
| Festuca summilusitana | Poaceae | Autumn | Autumn | 17.0 |
| Helictochloa marginata | Poaceae | Spring | Summer | 34.6 |
| Jurinea humilis | Asteraceae | Autumn | Winter | 191.2 |
| Luzula caespitosa | Juncaceae | Autumn | Winter | 107.0 |
| Minuartia recurva | Caryophyllaceae | Autumn | Autumn | 54.2 |
| Neoschischkinia truncatula | Poaceae | Autumn | Autumn | 17.6 |
| Phalacrocarpum oppositifolium | Asteraceae | Autumn | Autumn | 2.7 |
| Phyteuma hemisphaericum | Campanulaceae | Spring | Spring | 10.1 |
| Plantago holosteum | Plantaginaceae | Autumn | Autumn | 8.9 |
| Sedum anglicum | Crassulaceae | Autumn | Autumn | 62.9 |
| Sedum brevifolium | Crassulaceae | Autumn | Autumn | 28.4 |
| Sempervivum arachnoideum | Crassulaceae | Spring | Summer | 55.3 |
| Silene ciliata | Caryophyllaceae | Spring | Spring | 30.5 |
| Solidago virgaurea | Asteraceae | Autumn | Winter | 164.6 |
| Spergula morisonii | Caryophyllaceae | Autumn | Autumn | -62.1 |
| Thymus praecox | Lamiaceae | Autumn | Autumn | 65.8 |

Fig 1. Map of the study sites in northwest Spain, within the Cantabrian Mountain and the location of our two systems: Mediterranean (red) and Temperate (blue). Boxplots show respectively Mean annual air temperature (bio1) and Summer precipitation (bio17) calculated from CHELSA 2.1 (averages from 1981 - 2010) based on 47 locations per each study site.

Interfaz de usuario gráfica, Aplicación

Descripción generada automáticamente

Fig 2A. Weekly means of soil temperatures (Tmax and Tmin at 5 cm depth) measured during 10 years in two alpine sites of the Picos de Europa National Park, Cantabrian Mountains, Spain. Image of MLog5W, Geoprecision datalogger used in the field. 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.

Diagrama

Descripción generada automáticamente

Fig 3A. Cumulative germination curves, based on all target species sowed from each system 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. Density plots within each system represents the calculated area between individual species cumulative germination curves. At the bottom horizontal bars represent the phenology periods calculated. Fig 3B. Show cumulative germination curves for each individual species in the fellfield incubator from both systems. Fig 3C. Show cumulative germination curves for each individual species in the snowbed incubator from both systems.

Imagen que contiene Diagrama

Descripción generada automáticamente

Fig 4. Exploratory principal component analysis with 4 data subsets, system x incubator. Explanatory variables are the four germination phenology traits and EHS (Environmental heat sum)

Interfaz de usuario gráfica, Diagrama

Descripción generada automáticamente

Fig 5. Phenology traits responses to incubator chambers representing fellfield and snowbed microclimatic scenarios from Mediterranean and Temperate alpine systems 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 systems. Notice the different scales in y-axis in 4B. 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.

Gráfico, Gráfico de barras, Gráfico de rectángulos

Descripción generada automáticamente