# Title: Phenological germination shifts of alpine plants in contrasting microclimates.

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## Summary (max 200 words) (now: 203 words)

Research conducted: Understanding seed germination phenology is important to predict the survival and resilience of plant communities to climate change; yet little is known about the influence of microclimatic conditions on germination phenology.

Methods: We conducted a continuous germination seasonal experiment with fresh seeds to investigate the germination phenology of 54 co-occurring species in two alpine systems. Using long-term data collected in alpine soils we mimicked two contrasting microclimatic scenarios: (1) exposed edges with snow-free conditions and warmer temperatures (“fellfield2), and (2) micro-valley conditions with lengthy snow cover and cooler temperatures (“snowbed”).

Key results: We found a consistent germination shift, i.e. a temporal delay in snowbed compared to fellfield conditions in both study systems. Experimental germination results were validated by the germination phenology registered in the field. Despite macroecological effects, resulting in lower dormancy and higher autumn germination in the Mediterranean than in the temperate system, all the traits related to germination phenology showed similar responses under both microclimatic scenarios.

Main conclusion: These results suggest a generalizable phenological germination response of alpine plants to microclimatic conditions regulated by snow accumulation along microtopographical gradients. In a warming scenario with reduced snow cover and higher temperatures, alpine species are expected to anticipate germination with disrupting effects on communities dominated by cold-adapted species.

Keywords: alpine microhabitats, climate change, germination phenology, germination shift, microclimatic conditions, reproductive phenology.

## 1. Introduction

Plant phenology can be defined as the study of annually cyclical patterns of growth and developmental stages (Hopp, 1974). Particularly, plant reproductive strategies and their phenology have strong fitness implications for communities' evolvement and survival (Donohue, 2005; Poschlod *et al.*, 2013). Most studies focused on reproductive phenology have centred on flowering time, seed maturation and dispersal onset, describing a fast-slow continuum of reproductive phenology (Segrestin *et al.*, 2018; Segrestin, Navas and Garnier, 2020). Comparatively, few studies have focused on germination phenology although germination is a sensitive and irreversible process fundamental for plant regeneration (Baskin and Baskin, 2014). Previous experiments found that early season germination can benefit individuals with longer growing seasons (Donohue *et al.*, 2010) and give a competitive edge to limiting resources (Verdú and Traveset, 2005) against individuals germinating later during the season. However, early germination also involves higher mortality risks (Thomson, King and Schultz, 2017) due to warm spells or frost events compared to a more conservative strategy i.e. seeds germinating later in the season (ten Brink, Gremer and Kokko, 2020). Thus, germination timing proved to be key for successful regeneration and seedling survival (ref) as well as to be influential for population and community dynamics in response to changes in climatic conditions (Kimball *et al.*, 2011; Levine, Mceachern and Cowan, 2011; Huang *et al.*, 2016). There is a consensus that the germination process has evolved by adapting diverse approaches. One approach is to adopt a bet-hedging strategy, i.e. spreading the mortality risk with several germination episodes (Simons, 2011); another is to have specific environmental germination cues that trigger the germination process when abiotic conditions are favourable (i.e. temperature, moisture or light) (Donohue *et al.*, 2010; Baskin and Baskin, 2014) or a combination of both (Graham, Smith and Simons, 2014). Nonetheless, little is known about germination phenology in communities adapted to harsh and unpredictable environments which could help us understand how germination phenology may shift and adapt under current and future climate change scenarios.

Alpine areas display extreme and changing climatic conditions and are characterized by short growing seasons (Körner, 2021). Under these circumstances, germination phenology is of vital importance to match favourable conditions and to prevent unsuitable winter climate (Gremer *et al.*, 2020). The germination observed from species in these areas has been described in two general syndromes. One syndrome is called “global alpine germination syndrome”, derived mainly from temperate alpine species and characterized by a period of cold stratification followed by warm temperatures (Baskin and Baskin, 2014; Fernández-Pascual *et al.*, 2021). In the other syndrome, proposed for areas with Mediterranean-like climate, species germinate immediately after dispersal if water is available and under colder temperatures, and is referred to as “Mediterranean germination syndrome” (Giménez-Benavides, Escudero and Pérez-García, 2005). Nevertheless, germination strategies are highly variable in alpine ecosystems (Wagner and Simons, 2009) and the underlying causes of the variation observed deviating from the main germination syndromes are not fully known. 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) or species order of appearance in glacier foreland succession (Schwienbacher *et al.*, 2012). A worth considering particularity of alpine areas is their high topographic roughness which, at fines scales, translates into a mosaic of microclimatic conditions (Scherrer and Körner, 2011) with sharp temperature and snow-melting gradients (Körner, 2021) over a few centimetres (Graham *et al.*, 2012). 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 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 time. These experimental designs barely mimic real field climatic conditions and fluctuations, especially in the alpine environment (see “paper Picos JVS”) and do not consider the whole spectrum of germination phenology. To draw more robust conclusions about real-life germination phenology 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. To our knowledge, only one paper has tried to replicate natural environmental conditions, at two-weeks/monthly resolution, throughout a whole year in the Australian alps and were able to describe three different germination strategies (Hoyle et al. 2015).

The approach we present here is to study germination phenology across a whole year with continuous germination checks in two experimental field based and contrasting microclimatic conditions. Using microclimatic long-term data collected in alpine soils we mimicked two contrasting microclimatic scenarios. One scenario mimicked 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 and longer growing seasons; and the other mimicking micro-valleys (referred to as “snowbed scenario”) i.e., areas with dense plant cover, long snow cover and cooler and shorter growing seasons. The specific questions we want to investigate are: (1) will microclimatic scenarios modify the germination phenology of alpine grassland communities? And (2) do alpine grassland communities from different systems (with different macroclimates) show different patterns of germination phenology and respond differently to microclimatic conditions? Regarding our first question, we hypothesize that microclimatic scenarios will drive differences in the germination phenology. 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, in the temperate system, we expect more germination after cold stratification and under warmer temperatures; in concordance with the global alpine germination syndrome (Fernández-Pascual *et al.*, 2021); while in the Mediterranean system we expect higher autumn germination and germination at colder temperatures following the Mediterranean germination syndrome (Giménez-Benavides, Escudero and Pérez-García, 2005). We also expect that both systems respond similarly to the microclimatic conditions.

## 2. Material and Methods

## 2.1 Study systems

Our study focuses on alpine grasslands communities living between 1900 and 2500 m a.s.l. in the Cantabrian Mountains. This mountain range, running E-W in northern Spain, is considered a transitional biogeographical hub between Eurosiberian and Mediterranean regions (Jiménez-Alfaro *et al.*, 2021), influenced by Mediterranean-like climate in southern slopes and temperate climate in northern slopes. The particularity of its geographic location facilitates the coexistence of a complex array of communities with species adapted to both climates. We established one study system in each contrasting macroclimate: (1) temperate alpine calcareous grasslands in the Picos de Europa National Park (NE of the Cantabrian mountains, Fig.1A in green); and (2) Mediterranean high mountain acidic grasslands in the Valles de Omaña and Luna Biosphere Reserve (SW of the Cantabrian mountains, Fig.1a in yellow). Alpine grasslands communities in the Cantabrian Mountains are dominated mostly by *Poaceae* and *Cyperaceae*, and lifeform are mainly Hemicryptophytes and Chamaephytes. Grazing impact is restricted to wild populations of Cantabrian chamois (*Rupricapra pyrenaica parva*) in both systems. The temperate system presents higher richness (n= 8 - 18) with some relict alpine species from diverse origins: Eurosiberian, Mediterranean or endemic (Jiménez-Alfaro *et al.*, 2014) while local alpine diversity is regulated by meso-topographic gradients. The temperate 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 (based on eight locations, soil values recorded from 2008 to 2019, data not shown). The Mediterranean system, with lower plant richness (n= 3-14), is located above an acidic bedrock (pH 3.8 – 4.8, own data not shown). The system 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 (based on four locations, soil values recorded from 2021 to 2023, data not shown).

## 2.2 Seed sampling

Within each system we established four seed sampling sites, separated at least 500 m, assuming they form the same meta-population. In each sampling site, we recorded all vascular plant species co-occurring in a central representative plot of 3 m radius and considered them our target communities (49 species in the temperate and 22 species in the Mediterranean systems). During August-September 2021, we visited the sampling 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. Sampling took place within a 50 m radius of the central plot of each site from at least 20 – 50 randomly chosen individuals. Following standard protocols for sampling seeds of wild populations, the goal was to collect 200 seeds from each target species in two sampling sites, maximizing spatial sampling and intraspecific genetic diversity (ENSCONET 2009). In the temperate system, we obtained seeds from 38 target species, of which 19 were collected in two sites. In the Mediterranean system, we obtained enough seeds from 21 target species, covering two sampling sites for each species except in 2 cases. Only three species (*Jurinea humilis*, *Thymus praecox* and *Silene ciliata*) were present in both systems. Seed lots (species x sampling site, N = 95) remained in room conditions while we manually clean and sown each seed lot within 25 days of collection.

## 2.3 Germination trails

We did a sequential sowing of each species according to field collection, meaning that seeds entered the incubators at their optimum dispersal time. Each seed lot 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 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, no germination scores were done in that period. We removed seeds from the petri dishes once germination occurred (radicle > 1.5 mm long). At the end of the experiment, we cut open non-germinated seeds under the binocular loupe to visually assess if they were empty, infected, or seemed normal. We considered seeds with white and firm embryos viable, i.e. potentially germinable (Baskin and Baskin, 2014). We removed empty or infected seeds as well as populations with zero germination or with 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) for further analysis. The experiment was terminated after 14 months (July 2021 - September 2022) and the final dataset (Supporting information Table S1) 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.

To control for field germination phenology, we additionally sowed back 12 species, 6 from each system (Table 1), into two sampling sites. Within each sampling site we identified two areas with contrasting microclimatic conditions equivalent to our incubator scenarios, there we allocated 30 seeds from each species into mesh bags and buried them 3-5 cm deep in the soil. We sowed the seed bags in late September (Mediterranean system) and early October (temperate system) and programmed two retrieval times after winter period: one in early spring (one month within snowmelt) and one in late autumn (within one month of first snowfall). After retrieval, we counted the germinated seeds under the binocular loupe in the laboratory.

## 2.4 Microclimatic scenarios

We used the longest field soil temperature records in the temperate system to establish our microclimatic scenarios (10 years vs. 2 years for the Mediterranean system) which also showed the highest microclimatic variation between micro-valleys (snowbeds) and micro-edges (fellfields). We took 10 years of hourly temperature data from four sites (2008 - 2019 field data collected in alpine soils 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) (Fig. 1c). 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 (Zhang *et al.*, 2005). Then, we chose the two most contrasting sites in snow cover length and temperatures as climatic scenarios to recreate those same conditions in incubation chambers. The final experimental scenarios consisted of a weekly-resolution temperature program (Fig. 1d) with daily temperature ramps between daily Tmax and daily Tmin, and monthly-resolution photoperiod regimes. Each incubator 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). 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”. In the fellfield scenario we programmed winter period with below 0 ºC temperatures, and daily photoperiod and temperature fluctuation for 144 days. While in the snowbed scenario we programmed constant 0 ºC and darkness for 196 days. More detailed information about weekly programs is available in supporting information Table S2.

## 2.5 Germination traits

We defined a set of traits to describe germination phenology (Table 2) and to synthesize the diverse patterns observed during the continuous seasonal experiment, by considering their functional ecological significance in the alpine ecosystem. The first four traits contemplate the phenological season: autumn and summer germination consider specific periods of time measured in Julian calendar dates (i.e. equal length between incubators). Winter and spring germination are defined by specific thermal thresholds (i.e. different length between incubators). Winter germination period includes from the last germination score before winter (T mean < 3.5 ºC) until T mean > 2 ºC, from when the period changes to spring germination until summer solstice (germination score the 24th of June) (Fig. 2a bottom: 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 when reaching 50% germination (N = 68). Both measures were calculated by fitting a linear model between the two checks before and after reaching 50% germination to calculate the exact date and then compute the number days from sowing to T50. Finally, we considered total germination, calculated as the accumulated germination by the end of the experiment.

## 2.6 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 answer the first question, we analyzed each system separately and modelled, with MCMCglmm, every germination trait as response variable and incubator as explanatory variable (fixed factor), while phylogeny and sampling site (nested within species) were specified as random factors (germination trait~ incubator). To answer the second question, we tested each germination trait as response variable with 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 and Qian, 2019) and represented using phylosignal and phylobase R packages (Keck *et al.*, 2016; R Hackathon et al., 2020). 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 2 subsets, one per each incubator and perform a PCA with FactoMineR package (Le, Josse and Husson, 2008). To select non redundant traits, we first did a correlation analysis with corrplot (Wei and Simko, 2021) of all seven traits (supporting information Fig. S3), considering the high correlation between t50 and autumn germination (- 0.94) we decided to keep the later.

## 3. Results

## 3.1 Germination phenology patterns

The cumulative germination curves show a divergent pattern of germination phenology between incubators (Fig. 2a). Indistinctively of the system, fellfield scenario promoted germination earlier in the season, mainly in autumn or spring periods. On the contrary, snowbed incubator responses were modulate by the system and the germination peaks took place either in summer or autumn periods (for the temperate and Mediterranean systems respectively). The temperate system showed the highest germination period in spring for the fellfield scenario, while in snowbed scenario the germination peak was in summer. On the contrary, the Mediterranean system showed the highest germination in autumn period for both incubators.

Although these general patterns, both systems showed high interspecific variation between species specific germination responses. In fellfield scenario (Fig. 2b), the species from the temperate system showed germination peaks divided between autumn (31.6%) and spring period (44.7%) while in the Mediterranean species, we can observe a vast majority of the germination curves peaking during autumn period (71%). Meanwhile, in snowbed scenario (Fig. 2c), species of the temperate system changed their phenology and mostly peaked in summer period (36.8%); on the contrary, species of the Mediterranean system still germinated mainly in autumn period (57.1 %). Detailed individual species germination peak periods and delay to reach 50% germination can be checked in supporting information Table S4. The patterns observed in the continuous seasonal temperature laboratory experiment are authenticated by the field germination phenology we registered for 10 of our species, which showed compatible phenology patterns (Table 1, *Androsace villosa* and *Festuca glacialis* are not included because the laboratory germination experiment failed) and detailed individual graphs are shown in supporting information Fig. S5). In fellfield natural conditions, higher germination values were observed in the spring retrieval (germination could have happen the first autumn just after sowing or the first month after snowmelt). In snowbed natural conditions, higher germination values were observed in the autumn retrieval (the temperatures required were not reached until summer).

The delay observed between incubators germination curves confirms that microclimatic conditions generate a consistent germination shift. We measured this shift as the graphical space between both germination curves (one for each incubator) of every species (see individual species germination curves in supporting information Fig. S6). The temperate system showed a noticeable germination shift from the very beginning of the experiment (Fig. 2a), represented by the wider shape in the germination shift density plot, confirming that more species hard larger areas between germination curves i.e. bigger germination shift (Fig. 3a, left panel). On the other hand, the Mediterranean system showed a germination shift mainly after the winter period (Fig. 2a). Accordingly, density plot values are closer to 0, meaning that species had smaller germination shift between incubators (Fig. 3a, right panel). These results highlight that most species germinate better under warmer fellfield conditions. Nevertheless, the germination shift seems to be phylogenetically restrained as shown in Fig. 3b, with closely related species showing similar germination shifts in most cases.

### 3.2. Germination traits

We observed a consistent germination response pattern to microclimatic conditions in all our traits. 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. 4a: model size effects and Fig. 4b: mean values). Total germination was slightly higher in fellfield scenario in both systems, but differences were only significant in the temperate one. T50 values were consistently higher in snowbed scenario (Fig. 4b) and EHS did not statistically differ between scenarios (Fig. 4a). 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 germination shift (detailed germination trait values per species can be checked at supporting information Table S4).

The exploratory PCAs (Fig. 5) showed that species variability in the fellfield scenario responded concordantly in both systems. The first axis (explaining 42% of variation) is mainly driven by autumn germination while the second axis (27% of variation) is mainly explained by summer germ. Contrastingly, species variability in the snowbed scenario responded differently. The first axis (explaining 39% of variation) was mainly correlated to EHS and summer germination, the second axis (27% of variation) mainly explained by winter germination. (see detailed values of traits contributions and axis eigenvalues explanation in supporting information Table S7).

When comparing traits between systems we found significant differences suggesting different germination strategies (details of incubator \*system model in supporting information Table 8). As expected, autumn germination was significantly higher in the Mediterranean rather than in temperate system (Fig. 4b, 1st row). Germination during winter and summer periods did not differ between systems but in both cases, germination happened in the snowbed scenario (Fig. 4b, 2nd and 4th rows). Spring germination showed significant differences with higher values in the temperate system (Fig. 4b, 3rd row). At the end of the experiment, total germination was significantly higher in the Mediterranean system (Fig. 4b, 5th row). To conclude, the average T50 time was significantly higher in the temperate system (Fig. 4b, 6th row) and EHS was only significantly lower in species from the temperate system subjected to the snowbed scenario (Fig. 4b, last row). Corroborating the phylogenetical signal observed in the germination shift we found a strong phylogenetic signal, calculated by Pagel’s Lambda (Pagel, 1999), with similar patterns in both systems. All germination phenology traits had mean lambda values above 0.8 while traits related to germination speed (T50 and EHS) showed lower values around 0.4 (supporting information Table S8). In both systems, we found significant effects of phylogeny and intraspecific variability (random factors) for all germination phenology traits. In T50 and EHS traits we found lower or no significant effect of phylogeny while intraspecific variation had still a significant effect (supporting information Table S8).

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 than the rest (Fig. 6, note the different trajectory in germination phenology). Corroborating also the different drivers explaining species variability in PCAs.

## 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 and 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, Escudero and Pérez-García, 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 and 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 and Baskin, 2014; Fernández-Pascual *et al.*, 2021) corroborating the findings by many authors (Cavieres and 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, Marcante and Erschbamer, 2010).

The Mediterranean system followed the previously described Mediterranean germination syndrome (Giménez-Benavides, Escudero and Pérez-García, 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 and 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 and 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 and 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 and Mooney, 1968).

## 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, Morgan and Lord, 2013). Nevertheless, more research is needed to study species specific adaptations to microclimatic conditions, as well as their responses to limited water availability.

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## Competing interests

None declared.

## Authors contributions

CEA, EFP and BJA designed the research. CEA collected the data. CEA and EFP analysed the data. CEA wrote the first draft of the manuscript. All authors read and revised the manuscript and agreed on the final version.

## Data availability

The data that support the findings of this study is available in the Supporting Information of this article and GitHub together with R scripts.

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## Supporting information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Table S1 Raw germination data

Table S2 Detailed incubator programs

Table S3 Complete species germination traits

Table S4 PCA traits axis contributions

Table S5 Phylogenetic signals and random factors significances

Fig. S1 Germination traits correlation plots

Fig. S2 Field and laboratory germination comparison

Fig. S3 Individual species germination curves per sampling site and incubator

Methods S1 R code with comments

# Tables and figures

Table 1. Comparison of laboratory and field experiment highest germination peak for both microclimatic conditions.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| System | Species | Fellfield germination peak | | Snowbed germination peak | |
| Laboratory | Field | Laboratory | Field |
| Mediterranean | *Armeria duriaei* | Autumn | 2nd Autumn | Autumn | 2nd Autumn |
| *Dianthus langeanus* | Autumn | 1st Autumn  Spring | Autumn | 1st Autumn  Spring |
| *Luzula caespitosa* | Autumn | 1st Autumn  Spring | Spring | 1st Autumn  Spring |
| *Phyteuma hemisphaericum* | Spring | Spring | Summer | Spring  2nd Autumn |
| *Plantago holosteum* | Autumn | Spring  2nd Autumn | Autumn | Spring  2nd Autumn |
| *Silene ciliata* | Spring | Spring | Summer | Spring  2nd Autumn |
| Temperate | *Armeria cantabrica* | Spring-Summer | Spring  2nd Autumn | Summer | Spring  2nd Autumn |
| *Carex sempervirens* | Spring | Spring | Summer | 2nd Autumn |
| *Gypsophila repens* | Autumn-Spring | 1st Autumn  Spring | Summer | None |
| *Jasione cavanillesii* | Spring | 1st Autumn  Spring | Summer | 2nd Autumn |

Table 2: 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 3. Model incubator\*system results

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | Incubator | | System | | Incubator\*system | |
| Trait | L\_95% CI | U\_95% CI | L\_95% CI | U\_95% CI | L\_95% CI | U\_95% CI |
| Autumn | -1.68 | -0.93 | -5.27 | -1.60 | -2.18 | -0.94 |
| Winter | 4.36 | 6.32 | -5.43 | 0.64 | 0.02 | 5.65 |
| Spring | -2.01 | -0.96 | 0.72 | 3.60 | -2.54 | -1.04 |
| Summer | 1.30 | 2.52 | -0.09 | 2.29 | 0.47 | 2.06 |
| Total germination | -0.55 | 0.17 | -2.81 | -0.38 | -0.85 | 0.13 |
| T50 | 0.17 | 0.42 | 0.40 | 1.08 | 0.21 | 0.59 |
| EHS | -0.33 | 0.01 | -0.02 | 0.82 | 0.00 | 0.53 |

Fig. 1 Study system description. (a) Map of the study sites in northwest Spain, within the Cantabrian Mountain and the location of our two systems: Mediterranean (yellow) and Temperate (green). (b) 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. (c) 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. (d) 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 C.

Gráfico

Descripción generada automáticamente

Fig. 2 Representation of the cumulative germination curves of our two study systems. (a) Cumulative germination 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. (b) Cumulative germination curves for each individual species in the fellfield incubator from both systems. (c) Show cumulative germination curves for each individual species in the snowbed incubator from both systems.

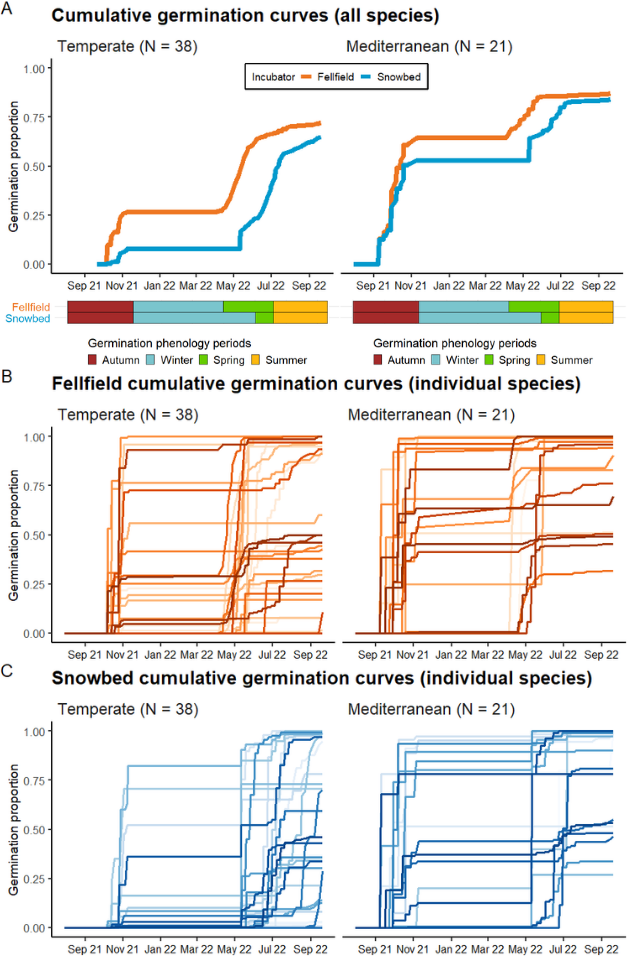


Fig. 3 Germination shift as response to microclimatic conditions. (a) Density plot in representation of the germination shift for each system. (b) Phylogenetical tree with each species calculated germination shift. Orange bars represent germination shift towards early season (higher germination in fellfield incubator in comparison to snowbed incubator, i.e bigger differential area), blue bars represent germination shift towards late season (higher germination in snowbed incubator in comparison to fellfield incubator).

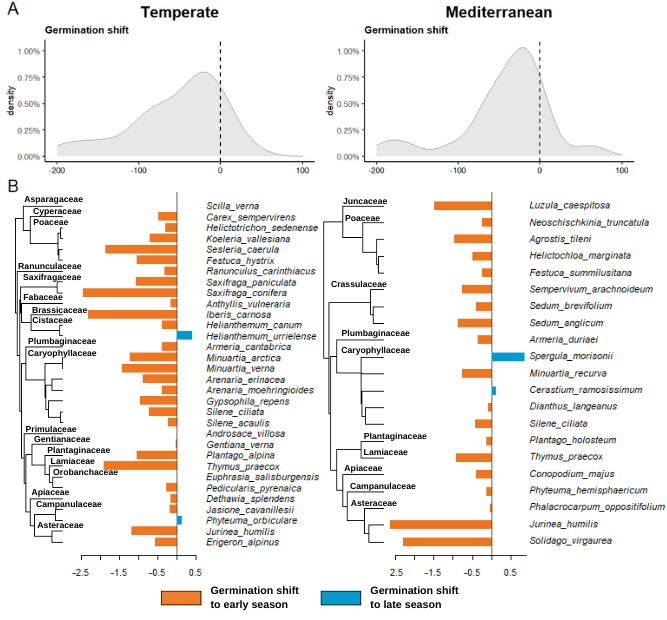


Fig. 4 Germination phenology traits responses for both incubators in each systems. (a) Model size effects of incubator according to the MCMC-glmm analysis of the data. Dots indicate mean effect size and whiskers are 95 % credible intervals (CI). The vertical dashed line marks the zero effect: when the 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). (b) Mean germination values for the seven traits at each scenario for both systems. Notice the different scales in y-axis. 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

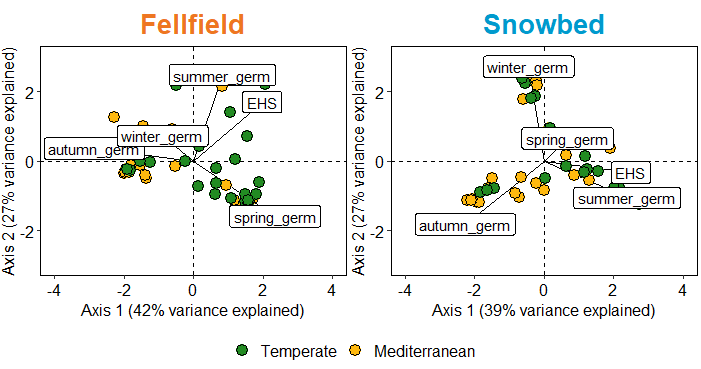
Fig. 5 Exploratory principal component analysis with 2 data subsets per each incubator. Explanatory variables are the four germination phenology traits and EHS.

Fig. 6 Relative germination proportion to each phenology period for both systems in our two incubators.

