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

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**Word counts**

Summary: 204 (200)

Introduction: 1238(1000)

Material and Methods: 1829 (1500)

Results: 726 (1000)

Discussion (30% of the total count): 1468 (1500)

Number of figures (indicate which figures in color): 6 (all in color)

Number of tables: 1

Supporting information:

## Summary (max 200 words) (now: 204 words)

* Understanding seed germination phenology is important to predict the survival and resilience of plant communities, yet little is known about the influence of microclimatic conditions on seed responses from seasonal ecosystems.
* We conducted a continuous seasonal experiment with fresh seeds to investigate the germination phenology of 54 species in two alpine systems influenced by temperate and Mediterranean climates. Using field microclimatic data series, we mimicked two contrasting scenarios: (1) exposed edges with snow-free conditions and warmer temperatures (fellfield), and (2) micro-valley conditions with lengthy snow cover and cooler temperatures (snowbed).
* The laboratory experiments were validated by germination phenology recorded in field sowing experiments. We found a germination delay in snowbed compared to fellfield conditions in the two study systems. Despite macroecological effects, resulting in lower dormancy and higher autumn germination in the Mediterranean than in the temperate system, all traits related to germination phenology showed similar responses under both microclimatic scenarios.
* Our results suggest a generalizable and quantifiable phenological shift in the germination of alpine plants along microclimatic gradients. In a warming scenario with reduced snow cover and higher temperatures, alpine species are expected to anticipate germination between 60 and 45 days, causing more disrupting effects on cold adapted species.

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

## 1. Introduction

Plant phenology informs about cyclical patterns of growth and developmental stages (Hopp, 1974) which are especially responsive to climate change (Scranton and Amarasekare, 2017). In seasonal climates, plant reproductive strategies and their phenology have strong fitness implications for communities' evolvement and survival (Donohue, 2005; Poschlod *et al.*, 2013). Studies focusing 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, very few studies have focused on seed germination phenology, despite this being a sensitive and irreversible process fundamental for plant regeneration (Baskin and Baskin, 2014). Early season germination can benefit individuals with longer growing seasons (Donohue *et al.*, 2010) and give a competitive edge to limiting resources against individuals germinating later during the season (Verdú and Traveset, 2005). 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 of seeds germinating later (ten Brink, Gremer and Kokko, 2020). Germination timing is therefore a key trait for regeneration and seedling survival, influencing population and community dynamics in response to climatic changes (Kimball *et al.*, 2011; Levine, Mceachern and Cowan, 2011; Huang *et al.*, 2016). Common adaptations of plants to regulate germination phenology include bet-hedging strategies that spread the mortality risk with several germination pulses (Simons, 2011); or responses to environmental cues that trigger the germination process under certain amount of temperature, moisture or light (Donohue *et al.*, 2010; Baskin and Baskin, 2014); and a combination of both (Graham, Smith and Simons, 2014).

The environmental regulation of germination phenology in largely unexplored in natural ecosystems, and most studies have focused on annual species from unpredictable water-dependent communities (Kimball *et al.*, 2011; Gremer and Venable, 2014; Thomson, King and Schultz, 2017; ten Brink, Gremer and Kokko, 2020). However, changes in germination phenology are also expected in other systems influenced by seasonality and climate change (Walck *et al.*, 2011). One important example is found in alpine and arctic ecosystems, where seed production and germination are strongly influenced by climate warming (Mondoni, Jiménez-Alfaro and Cavieres, 2022). Alpine areas are characterized by short growing seasons and display changing climatic conditions at different spatial scales (Körner, 2021). Under these circumstances, germination phenology is of vital importance to match favourable conditions and to prevent unsuitable winter climate during seed regeneration (Gremer *et al.*, 2020). The germination of alpine species has been generally described by two general syndromes. The global alpine germination syndrome occurs mainly in temperate climates and it is characterized by a period of cold-wet stratification followed by warm temperatures (Baskin and Baskin, 2014; Fernández-Pascual *et al.*, 2021). The cold-wet stratification is a basic requirement for most alpine species due to their dormancy-alleviating properties and this process it is assumed to happen only under snow accumulation which additionally provides an important thermal insulation from freeze-thaw events (Decker *et al.*, 2003). Concordantly, post-winter germination has been strongly affected in snow manipulation experiments (Drescher and Thomas, 2013; Drescher, 2014). Another consideration is that, without snow protection, temperatures drop below zero and the development of freezing tolerance has a potential fitness cost for species (Agrawal, Conner and Stinchcombe, 2004) and germination has not been shown to happen. In addition to the global alpine syndrome, a Mediterranean germination syndrome has been proposed for high elevation areas with Mediterranean-like climates, in which species germinate immediately after dispersal if water is available, show improved germination after a cold-wet stratification period and prefer higher temperatures (Giménez-Benavides, Escudero and Pérez-García, 2005; Giménez-Benavides *et al.*, 2018). However, germination strategies of alpine species may change dramatically within the same region, as a response to local elevation gradients (Fernández-Pascual et al., 2017), light exposures (Wagner and Simons, 2009), bedrock typology (Tudela-Isanta, Fernández-Pascual, *et al.*, 2018; Tudela-Isanta, Ladouceur, *et al.*, 2018) or successional stages in e.g. glacier forelands (Schwienbacher *et al.*, 2012).

At the local scale, the high topographic roughness of alpine landscapes translates into a mosaic of microclimatic conditions (Scherrer and Körner, 2011) with sharp temperature and snow-melting gradients (Körner, 2021) even at distances of few centimetres (Graham *et al.*, 2012). The impact of the microclimatic variation on germination phenology is largely unknown, and the few studies that consider its effect on seed germination patterns show contrasting results. Comparing germination patterns between alpine specialists of snowbeds and fellfields, Shimono & Kudo (2005) found no differences in the response of 27 species to temperature and light in Japan. In contrast, Rosbakh *et al* (2022) found different germination responses to temperature among 72 species occurring along a snowmelt gradient from the Caucasus. This suggests that germination patterns in alpine landscapes may differ in systems representing different ecological gradients or regional features (e.g., ecological or evolutionary history). In addition, germination experiments are limited by the use of fixed temperature conditions in incubation chambers (e.g., 12-h cycles of 20/10 and 15/5 ºC), which are decoupled from the continuous temperature cycles occurring in nature. To draw more robust conclusions about real-life germination strategies in alpine areas we need habitat specific detailed climatic data (Shimono and Kudo, 2005) and accurate experimental settings mirroring real field conditions in the best way possible (Hoyle et al. 2015). This is especially relevant if we want to understand the phenological responses of seed germination under contrasting microclimatic conditions. In alpine habitats, the spatial and temporal variation of microclimate is strongly dependent on the length of snow cover (Jiménez-Alfaro et al. 2024), making it necessary to consider the seasonal variation of snow and related temperature cycles in phenologically-driven studies.

In this study, we investigate seed germination phenology in 54 species from two alpine systems influenced by Temperate and Mediterranean climatic conditions in the same ecoregion. Our main aim is to understand how microclimatic variation affects germination phenology and the potential implications of such responses to plant regeneration in alpine communities. We conducted a continuous seasonal experiment in the laboratory based on the recreation of hourly data series of temperature and snow measured in alpine soils from the same region. Our experimental approach focused on two contrasting microclimatic regimes: fellfield conditions occurring in open and exposed areas subjected to wind, freeze and thaw cycles without snow protection in winter, with warmer and longer growing seasons; and snowbed conditions occurring in areas with dense plant cover, long snow cover and cooler and shorter growing seasons. We complemented the laboratory data with reciprocal sowing experiments in the field conducted in snowbed and fellfield conditions for a subset of species. Firstly, we asked at what extent microclimatic contrasting conditions occurring in alpine systems modify germination phenology. We hypothesized that fellfield conditions will result in higher total germination, earlier germination, and lower or no germination during winter, while the snowbed conditions will show opposite patterns, with lower total germination, later germination and germination under winter (snow-covered) conditions. Secondly, we ask whether alpine species from different macroclimates show similar phenological responses to microclimatic conditions. We hypothesize that species from the temperate system will germinate better after cold stratification and under warmer temperatures, in concordance with the global alpine germination syndrome (Fernández-Pascual *et al.*, 2021); while the species from the Mediterranean system will germinate mainly in autumn and at colder temperatures, following the Mediterranean germination syndrome (Giménez-Benavides, Escudero and Pérez-García, 2005). However, it is unclear whether the individual responses of alpine species will follow these syndromes homogenously, and whether germination phenology tested in the field will align with the results obtained in the laboratory.

## 2. Material and Methods

## 2.1 Study systems

We studied alpine grassland communities between 1900 and 2500 m a.s.l. in the Cantabrian Mountains, a mountain range running E-W in northern Spain, forming a transitional biogeographical hub between Eurosiberian and Mediterranean regions in Europe (Jiménez-Alfaro *et al.*, 2021). The biogeographic location of the region facilitates the coexistence of climatically temperate alpine communities and Mediterranean alpine communities in northern and southern slopes of the mountain range, respectively. We selected one study system for each 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 alpine acidic grasslands in the Valles de Omaña and Luna Biosphere Reserve (SW of the Cantabrian mountains, Fig. **1a** in yellow). The temperate system shows higher plant diversity, soil pH is 6.5 - 6.8 (own data), mean annual air temperature is 2.5 ºC and 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 5.7 ºC (based on eight locations, soil values recorded from 2008 to 2019, data not shown). The Mediterranean system supports lower plant richness and is located on acidic bedrock with pH 3.8 – 4.8 (own data). 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). The two study systems are moslty dominated by *Poaceae* and *Cyperaceae*, and the main lifeforms are Hemicryptophytes and Chamaephytes. Grazing impact is restricted to wild populations of Cantabrian chamois (*Rupricapra pyrenaica parva*).

## 2.2 Seed sampling

Within each study system, we established four seed sampling sites separated a minimum of 500 m from each other, maximizing the topographic diversity and the expected microclimatic variation among sites. 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 system). 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) from the target species. 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 Experimental setting

We used a temperature data series collected in the sites of the Temperate system (Picos de Europa National Park) from 2008 to 2019 as a reference for regional alpine microclimate. The data were collected with M-Log5W loggers, GeoPrecision, Ettlingen, Germany, accuracy +/- 0.1 ºC at 0 ºC and 0.01 ºC resolution. We selected the most contrasting field sites, representing snowbed and fellfield conditions (Fig. **1c**), which were further validated with temperature data available from the Mediterranean sites, based on a field data series from 2020 to 2022 collected with SP3 loggers, EMS, Czech Republic, accuracy +/- 0.1 ºC at 0 ºC and 0.01 ºC resolution. Despite differences in the total snow cover and mean temperatures, the selected snowbed and fellfield conditions were representative of the patterns observed in the two systems. We transformed the two reference data series for calculating maximum (T max) and minimum daily temperatures (T min) and calculated the number of days with snow cover, estimated as days with less than 1 ºC temperature variation around zero degrees (Zhang *et al.*, 2005). These conditions were then used to setup a weekly-resolution temperature program in two incubator chambers to conduct a continuous seasonal experiment (Fig. **1d**) and using monthly-resolution photoperiod regimes aimed to mimic field conditions. The chambers were programmed with daily temperature ramps between the Tmax and Tmin observed in the field. Each incubator was configured in Aralab incubators (Aralab climatic chamber Fitoclima S600 PL, equipped with 4 led modules 11W 350mA) using Fitolog 9000 software (version 9308, Aralab Pharmaceutical Stability software). The “fellfield” incubator was programmed with a snow-free winter period below 0 ºC temperatures with daily photoperiod and temperature fluctuation for 144 days. The “snowbed” scenario was programmed with a long snow period with constant 0 ºC temperature and darkness for 196 days. More detailed information about weekly programs is available in supporting information Table **S1**.

Germination experiments started sequentially after field collection of ripened seeds. Each seed lot was placed in both incubators simultaneously, sowing four replicates of 25 seeds in 9 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 3 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 period, 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 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). The final dataset (Supporting information Table **S2**) consisted of the raw scoring data for 95 seed lots of 54 species from 21 taxonomic families. A total of 10,816 viable seeds were used in the experiment.

## 2.4 Germination traits

We defined seven traits to describe germination phenology (Table **1**) and to synthesize the patterns observed in the experiments, also 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, and they are equal between incubators. Winter and spring germination are defined by specific thermal thresholds, and they differ between incubators; winter germination period includes from the last germination score before winter (T mean < 3.5 ºC) until T mean > 2 ºC, and spring germination includes from T mean >2ºC until summer solstice (24th of June) (Fig. **2a** bottom). We also calculated two traits related to germination speed: time to reach 50% germination (T50) and Environmental heat sum (EHS) to reflect how many days and accumulated degrees (in ºC) 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 to compute the number days from sowing to T50. We finally considered total germination, calculated as the accumulated germination at the end of the experiment.

## 2.5 Field sowing

We complemented the laboratory experiments with the sowing of seeds for monitoring germination phenology in the field. For each study system, we selected six species which are relatively dominant in the communities and represent different taxonomic families. The seeds were sown in two sampling sites for each study system, selecting local microclimatic conditions representing snowbed and fellfield conditions within each site. To do this, we used temperature data from an additional sampling of 20 data loggers (Thermochron, iButton, Newbury, UK; accuracy: +/- 0.5 ºC from -10 ºC to +65 ºC, resolution: 0.5 ºC, records every 4 hours) placed in the surroundings of each target community from 2021 to 2022. The two microclimatic conditions were easily recognizable from these data since snowbed microsites showed long snow cover and fellfield microsites had freezing temperatures in winter. In each microsite (n= 8; 2 conditions x 2 sites x 2 systems), 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) when temperatures are low enough to not trigger germination 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 and estimated their germination timing in the field.

## 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 study system separately and modelled, with MCMCglmm, every germination trait as response variable and incubator as explanatory variable (fixed factor), using phylogeny and sampling site (nested within species) as random factors (germination trait~ incubator). To answer the second question, we also 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 (Supporting information Fig. **S1**), created with V.PHYLOMAKER R package (Jin and Qian, 2019) 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). Field germination was analyzed using GLM with binomial family, separately for each species and retrieval time (spring and autumn) (field germination ~ microhabitat buried).

## 3. Results

## 3.1 Phenological patterns

The cumulative germination curves obtained from the experiment showed a divergent pattern between incubators (Fig. **2a**). Indistinctively of the system, fellfield conditions promoted germination earlier and the germination in snowbed conditions was delayed. The temperate system showed the highest germination period in spring for the fellfield scenario, while in the snowbed scenario the germination peak was in summer. On the contrary, the Mediterranean system showed the highest germination in autumn for both incubators. The two study systems showed a certain degree of interspecific variation. In the fellfield conditions (Fig. **2b**), species from the temperate system mainly showed germination peaks in autumn (31.6%) and spring (44.7%) while in the Mediterranean species, a majority of the germination curves peaked in autumn (71%). Meanwhile, in the snowbed conditions (Fig. **2c**), species of the temperate system changed their phenology and mostly peaked in summer (36.8%), while in the Mediterranean system most species kept germinating in autumn (57.1 %). Detailed individual species germination peak periods and delay to reach 50% germination are shown in supporting information Table **S3**.

The delay experienced by each species was measured as a phenological germination shift, i.e., the space between germination curves of each incubator (species germination curves are provided in supporting information Fig. **S2**). In a potential scenario of fellfield at the expense of snowbed conditions, most species anticipate their germination, indicated by negative values in the germination shift (Fig. **3a**). The only exceptions were two annual species (*Spergula morisonii* and *Cerastium ramossisimum*) and the local endemic *Helianthemum urrielense*. The other species differed in their degree of germination shift, with phylogenetically related species showing similar values in most cases (Fig. **3b**).

## 3.2. Phenological traits

For all traits, we found the same germination responses to microclimatic conditions in the two study systems (Fig. **4**). The fellfield conditions produced significantly higher autumn and spring germination, while the snowbed conditions promoted germination in summer and winter. Total germination was slightly higher in the fellfield conditions, but differences were only significant in the temperate system. EHS did not statistically differ between conditions (Fig. **4a**) and T50 values were consistently higher in the snowbed incubator (Fig. **4b**). The effect sizes were relatively larger in the temperate system, meaning that the differences between scenarios were stronger, in agreement with the observed germination shifts (detailed germination trait values per species are shown in supporting information Table **S3**).

When comparing traits between systems, we found significant differences in most cases (details of incubator\*system model size effects in supporting information Fig. **S3**). As expected, autumn germination was significantly higher in the Mediterranean rather than in the temperate system. Germination during winter and summer did not differ between systems, but in both cases the seeds only germinated in the snowbed conditions. Spring germination showed significant differences with higher values in the temperate system. At the end of the experiment, total germination was higher in the Mediterranean system. The average T50 and EHS were higher in the temperate system. In most traits we also found a significant interaction term between incubator and system (except in winter and total germination), showing that species from the temperate system in snowbed conditions responded more distinctively, either with higher germination values (summer) or lower germination values (autumn) than the rest. According to Pagel’s Lambda, we found a strong phylogenetic signal in both systems (supporting information Table **S6**). All traits had mean lambda values above 0.8, while traits related to germination speed (T50 and EHS) showed lower values around 0.4. Additionally, we found significant effects of phylogeny and intraspecific variability (random factors) for all traits. In T50 and EHS traits we found lower or no significant effect of phylogeny, while intraspecific variation had still a significant effect.

## 3.3. Germination in the field

The germination phenology recorded in the field also show a consistent delay between microhabitats (Fig. 5) in agreement with the results obtained in the laboratory. For 8 of the 12 species, spring retrieval show more seeds germinated in fellfield conditions, while in autumn we found more germinated seeds in snowbed conditions (although not all differed statistically, details in supporting information?). In three species (*Luzula caespitosa*, *Phyteuma hemisphaericum* and *Plantago holosteum*) spring retrieval showed more seeds germinated in snowbed conditions and only one species (*A. duriaei*) only showed germination in autumn retrieval.

## 4. Discussion

## 4.1 Microclimatic effects on germination phenology

Microclimatic scenarios drove significant differences in the germination phenology of the studied species. The fellfield scenario (mirroring micro-edges) prompted higher germination in early season, higher total germination, and almost no germination during winter. Conversely, in snowbed scenario (mirroring micro-valleys) we observed higher germination in late season, lower total germination, and higher germination in winter. The differences observed both in the laboratory and field experiments confirmed that microclimatic conditions along snowmelt gradients are an important source of variation in alpine germination phenology, in agreement with results found by Rosbakh *et al.*, (2022). Small microclimatic differences of two or three degrees Celsius in the lab resulted in high accumulated germination differences throughout the year. The differences here are shown as a phenology delay which potentially have detrimental effects on plant demography since fecundity fitness and seedling survival are tightly related to the start and length of the growing season (Donohue, 2005; Poschlod *et al.*, 2013).

In micro-edges (fellfield conditions), we observed two germination peaks. The autumn peak, immediately after sowing, 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 (Donohue, 2002), 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. As expected, below zero temperatures showed consistent detrimental effects on germination, a possibly explanation 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 thaws. This postponed germination could potentially shorten their effective growing season; nevertheless, this delay is compensated by the shorter winter period experienced in these areas. The second germination peak is triggered when temperatures rise again in early growing season and almost no germination happens in late growing season. However these results are in opposition to a study by Dürr *et al.*, (2015) which found that species inhabiting warmer soils tends to have a higher base temperature for germination (i.e. germinate later in the season), 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). The recorded field germination corroborated higher germination in early season compared to late season in fellfield conditions.

In micro-valleys (snowbeds conditions) germination peaks occurred either under snow or later in the season. Micro-valley conditions reduced autumn germination, partly because the lower temperatures experienced and seed dormancy constraints. Contrastingly, the winter germination peak demonstrate that some alpine species 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 restricted conditions are using the full extent of their already short growing season (Körner, 2021). After cold/wet stratification, species with warm temperature requirements are not fulfilled until late growing season, in concordance with (Rosbakh *et al.*, 2022), when we observed the second germination peak. In the field germination snowbed conditions didn’t show consistent pattern probably because we are observing two different processes: (1) in early season we recorded the germination that happened under snow while (2) in late season we recorded the germination of those species with warm temperatures requirements.

All species show a considerable intraspecific variability or germination plasticity to adapt to variable snow and temperature regimes, this ability may be a key point to successful regeneration under climate change scenarios. Considering the high levels of germination reached in our study, germination does not seems to be a bottleneck for successful regeneration and thus other stages like seedling establishment and survival need to be considered more vulnerable and limiting stages for the regeneration of alpine plants (Mondoni, Jiménez-Alfaro and Cavieres, 2022). Seedling emergence must be perfectly timed to be successful. Germination before winter or in early spring can lead to seedling 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). Within communities, the high interspecific variability also suggests that there will be winner and losers in future climate scenarios. According to our results species with strict cold/wet stratification requirements, which are more abundant in the temperate system, will be more negatively affected by the reduction and even loss of snow cover (Frei *et al.*, 2018).

## 4.2 Macroclimatic influence on germination phenology

As we expected, the temperate system diverged significantly from the Mediterranean system. 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, 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 guarantees 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 lower 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 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). 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 as lowland Mediterraenan species (Mattana *et al.*, 2022). The higher values of total germination reached in the Mediterranean system suggest that its species might be more successful in generating viable seeds germinating within the first year, but also meaning that the 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 undescribed in distinct cold-limited biomes like tropical alpine or artic areas.

## 4.3 Novelty, limitations, and further research

Our study focused on germination phenology presents a novel assessment of seed germination traits for a whole community 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. The two systems differed also in bedrock potentially having a cofounding factor in our study, as germination traits might differ between siliceous and calcareous bedrock (Tudela-Isanta, Ladouceur, *et al.*, 2018), nevertheless the microclimatic variation showed the same patterns in both systems. Although the microclimatic scenarios were based in the temperate system field data; the high germination responses showed by the Mediterranean system suggests 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, suggest that water availability may potentially have a stronger influence in germination than temperature in specific areas (Rosbakh and Poschlod, 2015). Nevertheless, there is still many gaps as how water availability directly affects germination, especially in water-limited ecosystems.

## Conclusions

In future alpine climate scenarios, warming is happening faster above the tree line (Körner, 2021), snow is decreasing (Frei *et al.*, 2018) and there is a higher frequency of frost events (Gerdol *et al.*, 2013). Considering such scenarios, 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). The seemingly stricter germination requirements in species from temperate systems will no longer be met and germination phenology alterations could have major regeneration implications (Mondoni *et al.*, 2012) in snowbed conditions (Rosbakh *et al.*, 2022) likely reducing the amount of successful germination or leaving seedlings vulnerable to frozen damage without snow protection (Venn, Morgan and Lord, 2013). Nevertheless, a greater threat might come from the expanding micro-edge climatic conditions, consequently reducing the suitable area of the specialized species adapted to micro-valleys (Hülber, Bardy and Dullinger, 2011; Pickering *et al.*, 2014; Verrall, Green and Pickering, 2023).

## Acknowledgements

We are grateful to Sara Alberdi and Víctor González for their invaluable help in laboratory work. Fieldwork was conducted under Picos de Europa National Park, who gave permission to sample seeds in the protected area. This research was funded by the Spanish Research Agency (AEI/10.13039/501100011033), SEEDALP: Seed ecological spectrum of alpine plant communities (MCI-20-PID2019-108636GA-I00) and MCI-21-PRE2020-092874: Ayudas para Contratos Predoctorales para la Formación de Doctores - FORMACIÓN PERSONAL INVESTIGADOR - FPI - del Ministerio de Ciencia e Innovación -

## 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 Detailed incubator programs

Table S2 Raw germination data

Table S3 Complete species germination traits

Table S4

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.** 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. |

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.

Imagen que contiene Diagrama

Descripción generada automáticamente

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).

Diagrama

Descripción generada automáticamente

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

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Fig. 5 Germination phenology in field sowing experiment. Seed germinated in the field (max N = 60) in both microclimatic conditions considered (fellfield vs snowbed) at each removal time (spring vs autumn).

Diagrama, PowerPoint

Descripción generada automáticamente