## Abstract

1. In most terrestrial plants, regeneration depends on the ability of seeds to germinate at the most favourable climatic conditions. Understanding seed germination phenology is thus crucial for predicting plant responses to environmental changes. However, a substantial gap persists regarding how microclimatic conditions influence germination in seasonal ecosystems.

2. Here, we investigate the germination phenology of alpine plants in snow-related microclimates as a tool for predicting the resilience of plant communities to climate change. We conducted a continuous seasonal experiment with fresh seeds to investigate germination phenology in 54 co-occurring species from temperate and Mediterranean alpine communities. Using long-term field microclimatic data series, we precisely mimicked two contrasting microclimatic conditions: (1) windy exposed edges with snow-free period in winter and warmer temperatures in summer ( “fellfield”) and (2) sheltered areas with lengthy snow cover and cooler temperatures (“snowbed”) in growth chambers. We validated the laboratory results with field sowing experiments to provide a complete picture of germination phenology.

3. The analysis of phenology traits demonstrated that both communities displayed similar responses to microclimatic variation. Accumulated microclimatic differences of 2-3 degrees Celsius a week, across a whole year in the laboratory, resulted in a quantifiable germination phenology delay, with an average of 60 and 45 days in snowbed conditions. The results from climatic chambers under realistic microclimatic conditions were consistent with the germination phenology registered from field experiments. We also observed macroclimatic effects resulting in reduced dormancy and increased autumn germination in Mediterranean alpine species.

Synthesis. This study combines novel laboratory and field experimentation to tackle the understudied topic of germination phenology in habitats with sharp microclimatic gradients. Specifically, our findings suggest a predictable phenological shift in the germination of alpine plants along microclimatic gradients. In warmer conditions with reduced snow cover, alpine species are expected to advance germination 52 days on average, with potential disrupting effects on cold-adapted plant communities. This highlights the role of germination phenology to determine plant-environmental relationships in mid-latitude ecosystems, with strong impact on plant establishment and extinction risks under local microclimatic gradients.

**Keywords:** Alpine microhabitats, climate change, germination phenology, germination shift, microclimate, Mediterranean alpine, temperate alpine, reproductive ecology, reproductive phenology.

## Translated Abstract

1. En plantas terrestres, la regeneración depende de la habilidad de las semillas para germinar en las condiciones climáticas más favorables. Por tanto, entender la fenología de la germinación es crucial para predecir respuestas de las plantas a cambios ambientales. Sin embargo, hay una considerable falta de conocimiento sobre como las condiciones microclimáticas influencian la germinación en ecosistemas estacionales.

2. En este estudio, investigamos la fenología de la germinación de plantas alpinas en gradientes de nieve a través de una nueva metodología para poder predecir la resiliencia de las comunidades alpinas al cambio climático. Realizamos un experimento estacional continuo con semillas frescas para investigar la fenología de la germinación de 54 especies que coocurren en comunidades alpinas Mediterraneas y templadas. Usando series a largo plazo de datos microclimáticos de campo, simulamos de forma precisa dos condiciones microclimáticas contrastantes: (1) crestas expuestas al viento, con períodos sin nieve en invierno y temperaturas altas en verano (i.e. “fellfield”) y (2) áreas protegidas con un período largo de nieve en invierno y temperaturas frescas en verano (i.e. “snowbed”). Adicionalmente, validamos los resultados obtenidos en el laboratorio con experimentos de siembra en campo, para así, proveer una visión completa de la fenología de la germinación.

3. El análisis de los caracteres de fenología de la germinación mostraron que las dos comunidades exhiben respuestas similares de fenología a la variación microclimática. Nuestros resultados mostraron que pequeñas variaciones semanales de 2-3 grados Celsius, a lo largo de todo el año en los experimentos de laboratorio), resultaron en un retraso de la fenología de la germinación cuantificable, con promedios de entre 60 y 45 días en las condiciones de snowbed. Los resultados de las cámaras de germinación, bajo condiciones microclimáticas realistas, fueron consistentes con la fenología de la germinación registrada en los experimentos de campo. También observamos efectos macroclimáticos como una menor dormición y mayor germinación en otoño en aquellas semillas de la comunidad Mediterránea.

Síntesis. Nuestro estudio combina innovadora experimentación de laboratorio con datos de campo para abordar el estudio de la fenología de la germinación en áreas alpinas con abruptos gradientes microclimáticos. Los resultados sugieren una tendencia cuantificable en la fenología de la germinación debido a estos gradientes. En condiciones más cálidas y con poca nieve, se espera que las especies alpinas adelanten su germinación una media de 52 días, con potenciales efectos negativos en aquellas especies adaptadas a germinar en condiciones más frías. Nuestros resultados indican que la fenología en la regeneración es un proceso crucial para determinar las relaciones entre plantas y ambiente en zonas alpinas de latitud media, con importantes impactos en el establecimiento de las plantas y en los riesgos de extinción bajo condiciones microclimáticas locales.

## Introduction

Plant phenology informs about cyclical patterns of growth and developmental stages (Hopp, 1974) which are responsive to climate change (Scranton and Amarasekare, 2017). In seasonal climates, plant reproductive strategies and phenology have strong implications for species fitness, which in turn affect community composition (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, few studies have focused on germination phenology, despite being a sensitive and irreversible process fundamental for regeneration (Baskin and Baskin, 2014). Early season germination can benefit individuals with longer growing seasons (Donohue *et al.*, 2010) and give a competitive edge in the use of limited resources versus individuals germinating later (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 delayed germination (ten Brink, Gremer and Kokko, 2020). Germination phenology is therefore a key trait for regeneration, influencing population and community dynamics in response to environmental change (Kimball *et al.*, 2011; Levine, Mceachern and Cowan, 2011; Huang *et al.*, 2016). Common adaptations to regulate germination phenology include bet-hedging strategies that spread mortality risk with several germination pulses (Simons, 2011); responses to environmental cues that trigger germination under a certain amount of temperature, moisture or light (Donohue *et al.*, 2010; Baskin and Baskin, 2014); or a combination of both (Graham, Smith and Simons, 2014).

Germination phenology has been studied in 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), but environmental regulation of germination phenology is 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 microclimatic conditions (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 global alpine germination syndrome has been characterized by a general requirement of cold-wet stratification followed by warm temperatures (Fernández-Pascual *et al.*, 2021). This cold-wet stratification has dormancy-alleviating properties and it is assumed to happen under snow, which additionally provides thermal insulation from freeze-thaw winter events (Decker *et al.*, 2003). Post-winter germination has been strongly influenced by snow manipulation experiments (Drescher and Thomas, 2013; Drescher, 2014) possibly because with no snow protection, temperatures drop below zero and the development of freezing tolerance has a potential fitness cost for species (Agrawal, Conner and Stinchcombe, 2004). In high-elevation areas with Mediterranean-like climates, species may follow a mediterranean germination syndrome by which seeds germinate immediately after dispersal if water is available, but also show enhanced germination with cold-wet stratification and relatively high temperatures (Giménez-Benavides, Escudero and Pérez-García, 2005; Giménez-Benavides *et al.*, 2018). Despite these general syndromes, germination strategies are known to vary in response to local elevation gradients (Fernández-Pascual et al., 2017), light exposures (Wagner and Simons, 2009), bedrock type (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 topographic heterogeneity of alpine landscapes translates into a mosaic of microclimatic conditions (Scherrer and Körner, 2011; Jiménez-Alfaro *et al.*, 2024) with sharp temperature and snow-melting gradients (Körner, 2021) even within few centimetres (Graham *et al.*, 2012). The impact of microclimatic variation on germination phenology is expected to be strong, but the few studies that have considered this showed contrasting results. By comparing germination patterns between alpine specialists of snowbeds (sheltered areas with snow accumulation, short growing season but low risk of frost and no water shortage) and fellfields (exposed areas where wind reduces the accumulation of snow resulting in soil freezing, frost damage and drought), Shimono & Kudo (2005) found no differences in the response of 27 alpine species to temperature and light in Japan. In contrast, Rosbakh *et al* (2022) found different germination responses to temperature among 72 species along a snowmelt gradient in 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). Nevertheless, germination experiments are limited by fixed temperature conditions commonly used 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 germination strategies in alpine areas, we need detailed climatic data (Shimono and Kudo, 2005) and accurate experimental settings mirroring real field conditions in the best way possible (Hoyle et al. 2015).

In this study, we investigate germination phenology of 54 alpine species influenced by either temperate or mediterranean climatic conditions within 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. By conducting a continuous seasonal experiment in the laboratory using temperature data series measured in the field, our experimental approach focused on two contrasting microclimatic regimes: (i) fellfield conditions occurring in open and exposed areas subjected to wind, freeze and thaw cycles without snow protection, with warmer and longer growing seasons; and (ii) snowbed conditions in areas with dense plant cover, long snow cover and cooler and shorter growing seasons. We complemented the laboratory data with field sowing experiments for a subset of species. Firstly, we asked at what extent microclimatic contrasting conditions modify germination phenology. We hypothesized that fellfield conditions will result in higher total germination due to longer growing season, earlier germination due to higher temperatures, and lower or no germination during winter due to below 0 temperatures. In contrast, the snowbed conditions will show lower total germination due to shorter growing season, delayed germination due to lower temperatures, and germination under winter (snow-covered) conditions due to temperature not dropping below 0 degrees Celsius. Secondly, we ask whether alpine species from temperate and Mediterranean climates show similar phenological responses. We hypothesized that species from the temperate community will germinate better after cold stratification and under warmer temperatures, in concordance with the global alpine germination syndrome; while the species from the Mediterranean community will germinate mainly in autumn and at colder temperatures, following the Mediterranean germination syndrome. 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.

## Material and Methods

### Study communities

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), thus facilitating the coexistence of climatically temperate and mediterranean alpine communities in the mountain range. We selected two study communities: (1) temperate alpine calcareous grasslands in the Picos de Europa National Park (NE of the Cantabrian mountains, Fig. **1a**); and (2) mediterranean alpine acidic grasslands in the Valles de Omaña and Luna Biosphere Reserve (SW of the Cantabrian mountains, Fig. **1a**). The temperate community shows higher species richness, soil pH is 6.3 - 6.8 (own data), mean annual air temperature is 2.5 ºC and mean summer precipitation is 260 mm(values extracted from Chelsa 2.1, bio 1 and bio 17 (Karger *et al.*, 2017), Fig. **1b**). Growing season starts in May until early October with a mean annual soil temperature of 5.7 ºC (soil values recorded from 2008 to 2019, data not shown). The mediterranean community supports lower plant richness and is located on acidic bedrock with pH 3.8 – 4.8 (own data). The community experiences a 2-month dry period in summer (average precipitation of 160 mm) 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 (soil values recorded from 2021 to 2023). The two communities are mostly 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*).

### Seed sampling

Within each study community, we established four sampling sites representing the dominant alpine vegetation type. The sites were separated at least 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 3 m radius plot and considered those our target species (49 species in the temperate and 22 species in the mediterranean community). 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 from at least 20 – 50 randomly chosen individuals. Following standard protocols for sampling seeds of wild plants, we aimed to collect 200 seeds from each target species in two sampling sites, maximizing spatial sampling and intraspecific genetic diversity (ENSCONET 2009). In the temperate community, we obtained seeds from 38 target species, of which 19 were collected in two sites. In the mediterranean community, 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 communities. Seed lots (species x sampling site, N = 95) remained in room conditions and were manually cleaned within 25 days of collection.

### Experimental setting

We used temperature data series collected in the sampling sites of the temperate community (Picos de Europa National Park) from 2008 to 2019 to calculate and average minimum and maximum temperature per day which we used as a reference for identifying regional alpine microclimates (M-Log5W loggers, GeoPrecision, Ettlingen, Germany, accuracy +/- 0.1 ºC at 0 ºC and 0.01 ºC resolution). We selected the most contrasting sampling 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 Micro-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 snowbed and fellfield conditions were representative of the patterns observed in the two communities. We transformed the two reference data series to 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 set up a weekly-resolution temperature program in two climatic chambers to conduct a continuous seasonal experiment (Fig. 1d) using monthly-resolution photoperiod regimes and daily temperature ramps between the Tmax and Tmin to mimic field conditions (see detailed climatic chambers programs in Table S1). The chambers were programmed with daily temperature ramps. Each condition was configured in one climatic chamber (Aralab Fitoclima S600 PL, equipped with 4 led modules 11W 350mA) using Fitolog 9000 software (version 9308, Aralab Pharmaceutical Stability software). The fellfield climate regime was programmed with a snow-free winter period below 0 ºC temperatures (ranging -0.5 to -2ºC) with daily photoperiod and temperature fluctuation for 144 days. The snowbed climate regime was programmed with a long snow period with constant 0 ºC temperature and darkness for 196 days.

Germination experiments started sequentially after field collection and seed cleaning. 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 period, incubators remained closed to avoid temperature increases that could trigger germination; 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 stereoscope to visually assess if they were empty, infected, or normal. We considered seeds with white and firm embryos viable (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 experimental conditions did not successfully 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 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.

### Germination traits

We defined seven traits to describe germination phenology (Table **1**) and synthesize the patterns observed in the experiments, also considering their functional ecological significance in the alpine ecosystem. The first four traits concern the phenological season: autumn and summer germination consider specific periods measured in Julian calendar dates, and they are equal between incubators. Specific thermal thresholds define winter and spring germination, and they differ between incubators; winter germination period covers 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. **2** 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 (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 predicted date and the number of days from sowing to T50. We finally considered total germination, calculated as the accumulated germination at the end of the experiment.

### Field sowing

We complemented the laboratory experiments with field sowing to represent germination phenology in natural communities. For each study community, we selected six species relatively dominant in the communities and represent different taxonomic families. The seeds were sown in two sampling sites for each community, in local microclimatic conditions identified as snowbed and fellfield 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 communities), we allocated 30 seeds from each species into mesh bags (3 replicate 10 seeds each) and buried them 3-5 cm deep in the soil. We sowed the bags in late September (mediterranean community) and early October (temperate community), when temperatures were 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 stereoscope in the laboratory and estimated their germination phenology in the field.

### Statistical analysis

We analysed 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 t50 and EHS we scaled the values and used Gaussian MCMCglmms (family = gaussian).

To answer the first question (i.e., at what extent microclimatic contrasting conditions modify germination phenology), we analysed each study community separately and modelled every germination trait as the response variable and climate regime (fellfield and snowbed) as the explanatory variable (fixed factor), using phylogeny and sampling site (nested within species) as random factors (germination trait~ climate regime). To answer the second question (i.e., whether alpine species from temperate and Mediterranean climates show similar phenological responses), we tested each germination trait as the response variable with both climate regime and community as explanatory variables (fixed factors) paying special attention to their interaction. Random factors remained the same as in the first model (germination trait ~ climate regime \* community). 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). Field germination was analysed using GLM with binomial family, separately for each species and retrieval time (spring and autumn) (field germination ~ microhabitat buried).

## Results

### Phenological patterns

The cumulative germination curves obtained from the experiment showed a divergent pattern between climate regimes (Fig. **2**). In both communities, fellfield conditions (with warmer temperatures) promoted germination earlier, clearly before winter in the temperate community and more prominently after winter in the mediterranean community, the germination in snowbed conditions was delayed. The temperate community showed the highest germination period in spring for the fellfield conditions, while in the snowbed conditions, the germination peak was in summer. On the contrary, the mediterranean community showed the highest germination in autumn for both climate regimes. The two study communities showed a certain degree of interspecific variation. In the fellfield conditions, species from the temperate community mainly showed germination peaks in autumn (31.6%) and spring (44.7%) while in the mediterranean species, most germination curves peaked in autumn (71%). Meanwhile, in the snowbed conditions, species of the temperate community delayed their phenology and mostly peaked in summer (36.8%), while in the mediterranean community most species kept germinating in autumn (57.1%), see supporting information Fig. S2. Detailed individual species germination peak periods and delay to reach 50% germination are shown in supporting information Table **S2**.

The delay experienced by each species was measured as the difference between reaching 50% germination and as a phenological germination shift, i.e., the space between germination curves of each climate regime (see species germination curves in supporting information Fig. **S3**). In a scenario with fellfield conditions compared to snowbed conditions, most species advanced their germination. On average, the advance in germination (T50) reached 60 days in the temperate community and 45 days in the mediterranean community. Concordant results were indicated by the negative values in the germination shift (Fig. **3a**). The only exceptions were two annual species (*Spergula morisonii* and *Cerastium ramossisimum*, both Caryophyllaceae) and the local endemic *Helianthemum urrielense* (Cistaceae). The other species differed in their degree of germination shift, with phylogenetically related species showing similar values in most cases (Fig. **3b**).

### Phenological traits

For all traits, we found similar germination responses to microclimatic conditions in the two study communities (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 community. Environmental heat sum (EHS) did not statistically differ between conditions (Fig. **4a**) and T50 values were consistently higher in the snowbed climate regime (Fig. **4b**). The effect sizes were relatively larger in the temperate community, meaning that the differences between conditions were stronger, in agreement with the observed germination shifts (detailed model results for each trait considering the communities separately are shown in supporting information Table **S3**).

When comparing traits between communities, we found significant differences in most cases (see details of climate regime\*community model size effects in supporting information Table **S4**). As expected, autumn germination was significantly higher in the Mediterranean rather than in the temperate community. Germination during winter and summer did not differ between communities, but in both cases, the seeds only germinated in the snowbed conditions. Spring germination showed significant differences with higher values in the temperate community. At the end of the experiment, total germination was higher in the Mediterranean community. The average T50 and EHS were higher in the temperate community. In most traits we also found a significant interaction term between climate regime and community (except in winter and total germination), showing that species from the temperate community in snowbed climate regime responded more distinctively, either with higher germination values (summer) or lower germination values (autumn) than the rest. According to Pagel’s Lambda (M. Pagel, 1999), we found a strong phylogenetic signal in both communities (supporting information Table **S5**). 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.

### Field germination

In agreement with the results obtained in the laboratory, the germination phenology recorded in the field showed a consistent delay between microhabitats (Fig. **5**). For 8 of the 12 species, spring retrieval showed more seeds germinated in fellfield conditions, while in autumn we found more germinated seeds in snowbed conditions (although not all differed statistically, see details in supporting information Table **S6**). In three species (*Luzula caespitosa*, *Phyteuma hemisphaericum* and *Plantago holosteum*) spring retrieval showed more seeds germinated in snowbed conditions, and in one species (*Armeria duriaei*) germination was higher in autumn retrieval.

## Discussion

### Microclimatic effects on germination phenology

Results of our experiments indicate that microclimatic conditions strongly influence germination phenology in the studied alpine species. The fellfield conditions prompted higher germination in the early season, higher total germination, and almost no germination during winter. Conversely, in the snowbed conditions we observed higher germination later in season, lower total germination, and higher germination in winter. Our results from the laboratory and field experiments confirmed that microclimatic conditions along snowmelt gradients are an important source of variation in germination, as previously suggested (Rosbakh *et al.*, 2022). Nevertheless, we acknowledge that, even though the detailed temperatures mimicked field temperatures, other environmental conditions like light, predation or drought were not considered. Despite this, we found that small microclimatic differences of 2-3 degrees Celsius in the lab resulted in a quantifiable phenology delay in snowbed condition, with an average of 60 and 45 days for the temperate and mediterranean communities respectively. This delay could 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 fellfield conditions, we observed two germination peaks. The autumn peak, immediately after sowing, can be seen as an opportunistic strategy as it provides a selective advantage for seedlings which can initiate development earlier (Donohue, 2002), but only if seedlings can survive winter conditions (Mondoni *et al.*, 2015). However, the success of surviving winter seems to be species-specific and more studies are needed to understand the implications of autumn germination. As expected, below-zero temperatures showed consistent detrimental effects on germination, likely driven by the physiological drought they are subjected to (Billings and Mooney, 1968). Consequently, seeds that disperse in fellfield conditions must endure below 0ºC 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 fellfield conditions. The second germination peak is triggered when temperatures rise again in the early growing season and almost no germination happens in the late growing season. The second peak was corroborated by our sowing experiments in the field, with higher germination in the spring compared to the second autumn in fellfield conditions.

In snowbed conditions, germination peaks occurred either under snow or later in the season, reducing autumn germination, likely because the seeds experienced lower temperatures and had dormancy constraints. The winter germination peak demonstrates that snow-like conditions (darkness and constant 0ºC) can break seed dormancy and some alpine species are able to germinate successfully in these conditions. Thus, species able to germinate under snow are using the full extent of their short growing season (Körner, 2021). After cold/wet stratification, warm temperature requirements for germination are not fulfilled until late growing season (Rosbakh *et al.*, 2022), when we observed the second germination peak. The adaptation to germinating at higher temperatures could prevent germination at dispersal time (Tudela-Isanta, Fernández-Pascual, *et al.*, 2018) since in autumn temperature drops rapidly in alpine areas. In the field experiments, snowbed conditions did not show consistent patterns probably because we were observing two peaks: in the early season we recorded the germination that happened under snow, while in the late season we recorded the germination of those species with warm temperatures requirements. We also point out that field germination showed slightly lower success (in both climatic conditions) than our laboratory results, reflecting the effect of other environmental conditions (predation, light availability or drought) on natural conditions.

We also found a considerable interspecific and intraspecific variability, or germination plasticity, in response to snow cover and temperature, an ability that may be crucial to successful regeneration under climate change. Considering the high germination levels reached in our study, germination does not seem to be a bottleneck for successful regeneration, and other stages like seedling establishment and survival need to be considered more vulnerable for the regeneration of alpine plants (Mondoni, Jiménez-Alfaro and Cavieres, 2022). However, 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); and with delayed germination, the underdeveloped root system of seedlings may be at risk in summer, when drought events can lead to death due to topsoil desiccation (Billings and Mooney, 1968). Within communities, interspecific variability in germination phenology also suggests that there will be winners and losers in future climate scenarios. Our results suggest that species with strict cold/wet stratification requirements, which are more abundant in the temperate community, will be more negatively affected by the reduction and even loss of snow cover (Frei *et al.*, 2018). Another worth considering point is that increasing autumn temperatures might also promote seed germination, potentially expanding the growing season but successful seedling survival will likely depend on winter conditions (Mondoni et al., 2012).

### Macroclimatic influence on germination phenology

As expected, the temperate community diverged significantly from the Mediterranean community. The temperate community followed the “alpine germination syndrome” (Fernández-Pascual *et al.*, 2021) with low germination before cold stratification and higher germination once the climatic chambers reach warm temperatures, corroborating previous findings (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). Delayed germination after winter guarantees a drought-free period during snowmelt, but the highest germination was observed once the temperatures rose above 12 ºC. In the fellfield conditions, the thermal threshold was surpassed earlier in the growing season while in the snowbed climate regime it was exceeded later in the growing season. The lower total germination (assuming high seed viability) registered in the temperate community suggests that some species might have a bet-hedging strategy, probably with fluctuating levels of dormancy and are more prone to form persistent soil seed banks (Schwienbacher, Marcante and Erschbamer, 2010).

The Mediterranean community showed the highest germination in autumn and earlier in the growing season, following the previously described “Mediterraneanmediterranean germination syndrome” (Giménez-Benavides, Escudero and Pérez-García, 2005). Accordingly, we found a higher proportion of non-dormant species (82%) whose seeds could germinate immediately after dispersal, when water was available. The second germination peak was observed in early growing season, suggesting that the studied species are well adapted to germinate at cooler temperatures than lowland Mediterranean species (Mattana *et al.*, 2022). The higher values of total germination reached in the Mediterranean community also suggest that these species might be more successful in generating viable seeds germinating within the first year, but also means that the soil seed bank might not be persistent. The significantly distinct patterns observed in our two communities potentially indicate the existence of other germination syndromes still undescribed in distinct cold-limited biomes like tropical alpine or arctic areas.

We note that the two study communities also differed in bedrock, potentially having a confounding effect, as germination traits might differ between siliceous and calcareous bedrock (Tudela-Isanta, Ladouceur, *et al.*, 2018). Nevertheless, the fact that the two communities showed the same germination responses to the microclimatic conditions suggests that the drivers of germination phenology are mainly linked to local snow-related. Although previous studies suggest that temperature is the main factor influencing germination phenology (Baskin and Baskin, 2014; Körner, 2021), the results of the mediterranean community, with species able to germinate at 5 ºC, suggest that water availability may potentially have a stronger influence than temperature in drought-sensitive systems (Rosbakh and Poschlod, 2015). Therefore, our results should be interpreted only in terms of temperature-related responses, and new studies are still needed to understand how water availability directly affects germination in alpine communities.

### Conclusion

By combining a continuous experimental approach for germination experiments with the delineation of phenological traits, this study provides new information about the influence of microclimatic variation on germination phenology in alpine ecosystems. Overall, our results suggest a generalizable and quantifiable phenological shift in the germination of alpine plants along microclimatic gradients related to soil temperature and snow cover. Germination phenology is therefore a plastic trait that can be anticipated or delayed by alpine microclimates, with potentially strong implications for the regeneration of plant communities (Mondoni *et al.*, 2012). In future climatic scenarios, alpine areas will suffer increasing warming (Körner, 2021), decreasing snow (Frei *et al.*, 2018) and higher frequency of frost events (Gerdol *et al.*, 2013). According to our results, this increasing warming climate will advance germination of alpine species 52 days on average, with potential disrupting effects like the mismatch of favourable conditions and a decrease of germination success on cold-adapted alpine species (e.g., *Helianthemum urrielense or Luzula caespitosa*) due to their strict cold/wet stratification requirements for germination. The physiological basis for such germination shift is expected to influence alpine species from temperate or Mediterranean communities in a similar way. However, the real impact of germination shifts will depend on individual species responses along microclimatic gradients of temperature and snow cover, and the potential of each species to adapt their phenological traits to new climatic conditions. Further studies also will need to combine the effects of germination shifts with the survival and establishment of seedlings along spatiotemporal changes in microclimatic conditions.

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## Figure Legends

**Figure 1.** Study community description. (a) Map of the study sites in the Cantabrian mountains, northwest Spain, and the two studied communities: mediterranean and temperate. (b) Boxplots show 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, with the image of MLog5W, Geoprecision datalogger used in the field. (d) Experimental temperature programs with weekly resolution, daily temperature ramps and monthly photoperiods representing fellfield and snowbed conditions in laboratory climatic chambers. Both climate regimes were configured based on real field data showed in C.

**Figure 2.** Representation of the cumulative germination curves of the two study communities throughout the germination experiment. Within each panel, orange and blue curves represent germination proportion in the fellfield and snowbed conditions, respectively. Flat areas represent cold period when no germination scores were done. Horizontal bars in the bottom represent the phenology periods calculated.

**Figure 3.** Germination shift in fellfield conditions compared to snowbed conditions in temperate and mediterranean communities. (a) Density plots show, for all the species in a community, the frequency distribution (y axis) of values (x axis) in germination shifts, calculated as the area-based differences in cumulative germination curves between fellfield and snowbed climatic regimes (provided in supporting information Fig. **S2**). (b) Phylogenetic tree and germination shift of the studied species. Orange bars represent the germination shift towards early season (earlier and higher germination in fellfield conditions, i.e., the longer the bar, the bigger the germination difference between climate regime), and blue bars represent the germination shift towards late season (later and higher germination in snowbed conditions).

**Figure 4.** Responses of germination phenology traits to climate regimes in temperate and mediterranean communities. (a) Model size effects of climate regime according to MCMC-glmm analysis. 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 condition (blue background), while dots on the left side of the zero-line (negative) mean higher values in the fellfield condition (orange background). (b) Mean germination values for the germination phenology traits. Notice the different scales in the y-axis. Error bars in germination phenology traits autumn, winter, spring, summer and total are binomial confidence intervals. Error bars in T50 and EHS are gaussian confidence intervals.

**Figure 5.** Germination phenology in field sowing experiment. The bars represent the number of seeds germinated in the field (max N = 60) in two microclimatic conditions (fellfield vs snowbed) at each retrieval time (early season vs late season) in the studied temperate and mediterranean communities.

**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 | Potential drawbacks |
| 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. | Risk of frost damage in case of punctual temperature drop as well as risk of drought. |
| Winter germination | From last germination score before winter, T mean < 3.5 ºC, until T mean > 2 ºC. Different length in each climate regime. | Germination of species able to germinate under snow-like conditions (zero degrees and darkness). Maximizes ability of taking advantage of water available after snowmelt for rapid seedling recruitment. | Risk of frost damage if snow is swept by wind or melt earlier. |
| Spring germination | Germination at the summer solstice relative to end of winter period (from T mean > 2ºC to summer solstice). Different length in each climate regime. | Germination with cold stratification requirement but no need for high temperatures. Rapid onset of germination following snowmelt. | Risk of frost damage in early spring. |
| 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. | Risk of drought stress before root system is properly developed. |
| 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 climate regime. |  |