## 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 to limited resources against 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). A global alpine germination syndrome has been described and 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 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 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). In high-elevation areas with Mediterranean-like climates, many species 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, little is known about germination phenology in temperate and Mediterranean alpine systems, where germination strategies are known to vary in 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 topographic roughness 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 it showed contrasting results. By comparing germination patterns between alpine specialists of snowbeds and fellfields, 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). In addition, germination experiments are limited using 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 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. We conducted 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, earlier germination, and lower or no germination during winter, while the snowbed conditions will show lower total germination, delayed germination and germination under winter (snow-covered) conditions. Secondly, we ask whether alpine species from temperate and Mediterranean climates show similar phenological responses. We hypothesized that species from the temperate system will germinate better after cold stratification and under warmer temperatures, in concordance with the global alpine germination syndrome; while the species from the Mediterranean system 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 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), thus facilitating the coexistence of climatically temperate and Mediterranean alpine communities in the mountain range. We selected two study systems: (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 system 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 kg·m2 (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 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 (soil values recorded from 2021 to 2023). The two systems 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 system, 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 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 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 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 and were manually cleaned within 25 days of collection.

### Experimental setting

We used temperature data series collected in the sampling sites of the temperate system (Picos de Europa National Park) from 2008 to 2019 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 systems. 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 incubator 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. The chambers were programmed with daily temperature ramps. Each incubator was configured in Aralab climatic chambers (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” incubator 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 loupe 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 contemplate 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. **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 (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 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 monitor germination phenology in natural communities. For each study system, we selected six species relatively dominant in the communities and represent different taxonomic families. The seeds were sown in two sampling sites for each system, 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 systems), 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 system) and early October (temperate system) 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 loupe 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 system separately and modelled every germination trait as the response variable and incubator as the explanatory variable (fixed factor), using phylogeny and sampling site (nested within species) as random factors (germination trait~ incubator). 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 incubator and system as explanatory variables (fixed factors) paying special attention to their interaction. Random factors remained the same as in 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). 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 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 conditions, while in the snowbed conditions, 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, most 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 **S1**.

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 incubator (species germination curves are provided in supporting information Fig. **S2**). In a scenario with fellfield conditions compared to snowbed conditions, most species anticipate their germination. On average, species from the temperate system advanced germination (T50) in 60 days while in the Mediterranean system was 45 days. 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*) 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**).

### Phenological traits

For all traits, we found similar 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. Environmental heat sum (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 conditions were stronger, in agreement with the observed germination shifts (detailed model results for each trait considering the systems separately are shown in supporting information Table **S2**).

When comparing traits between systems, we found significant differences in most cases (details of incubator\*system model size effects in supporting information Table **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 (M. Pagel, 1999), we found a strong phylogenetic signal in both systems (supporting information Table **S4**). 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.

### Germination in the field

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, details in supporting information Table **S5**). In three species (*Luzula caespitosa*, *Phyteuma hemisphaericum* and *Plantago holosteum*) spring retrieval showed more seeds germinated in snowbed conditions and only one species (*Armeria duriaei*) only showed germination in autumn retrieval.

## Discussion

### Microclimatic effects on germination phenology

Our experiments indicate that microclimatic conditions strongly influence germination phenology in 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). We showed that small microclimatic differences of two/three degrees Celsius in the lab resulted in a quantifiable phenology delay, with an average of 60 and 45 days in snowbed conditions for the temperate and Mediterranean systems 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 early season compared to the late season 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. Contrastingly, the winter germination peak demonstrates that some alpine species are adapted to germinate in snow-like conditions (darkness and constant 0ºC) and successfully break seed dormancy. Thus, species able to germinate in those conditions 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). In the field experiments, snowbed conditions didn’t show consistent patterns probably because we were observing the two peaks: (1) in the early season we recorded the germination that happened under snow, while (2) in the late season we recorded the germination of those species with warm temperatures requirements.

We also found a considerable 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 levels of germination reached in our study, germination does not seem 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). 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 postponed 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 system, will be more negatively affected by the reduction and even loss of snow cover (Frei *et al.*, 2018).

### Macroclimatic influence on germination phenology

As 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 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). Postponed 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 snowed conditions it was exceeded later in the growing season. The lower total germination registered in the temperate system 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 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%) 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 system 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 systems 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 systems also differed in bedrock, potentially having a confounding factor, as germination traits might differ between siliceous and calcareous bedrock (Tudela-Isanta, Ladouceur, *et al.*, 2018). Nevertheless, the fact that the two systems showed the same germination responses to the microclimatic conditions suggests that the drivers of germination phenology are mainly linked to fellfield and snowbed conditions. Another point worth considering is although literature agrees that temperature is the main factor influencing germination phenology (Baskin and Baskin, 2014; Körner, 2021), the results of the Mediterranean system, with species able to germinate at 5 ºC, suggest that water availability may potentially have a stronger influence in germination than temperature in specific areas (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 systems.

### 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 climatic trend might anticipate germination of alpine species 52 days on average, with disrupting effects on cold-adapted alpine species 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 systems 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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## References

Agrawal, A. A., Conner, J. K. and Stinchcombe, J. R. (2004) ‘Evolution of plant resistance and tolerance to frost damage’, *Ecology Letters*, 7(12), pp. 1199–1208. doi: 10.1111/j.1461-0248.2004.00680.x.

Baskin, C. C. and Baskin, J. M. (2014) *Seeds. Ecology, Biogeography and Evolution of Dormancy and Germination*. 2nd Editio, *Seeds*. 2nd Editio. San Diego, CA, USA: Academic Press. doi: 10.1016/B978-0-12-416677-6.00001-9.

Billings, W. D. and Mooney, H. A. (1968) ‘The ecology of arctic and alpine plants’, *Biological Reviews*, 43(4), pp. 481–529. doi: 10.1111/j.1469-185X.1968.tb00968.x.

ten Brink, H., Gremer, J. R. and Kokko, H. (2020) ‘Optimal germination timing in unpredictable environments: the importance of dormancy for both among- and within-season variation’, *Ecology Letters*, 23(4), pp. 620–630. doi: 10.1111/ele.13461.

Cavieres, L. A. and Arroyo, M. T. K. (2000) ‘Seed germination response to cold stratification period and thermal regime in Phacelia secunda (Hydrophyllaceae): Altitudinal variation in the mediterranean Andes of central Chile’, *Plant Ecology*, 149(1), pp. 1–8. doi: 10.1023/A:1009802806674.

Decker, K. L. M. *et al.* (2003) ‘Snow Removal and Ambient Air Temperature Effects of Forest Soil Temperatures in Northern Vermont’, *Soil Science Society of America Journal*, 67(5), pp. 1629–1629. doi: 10.2136/sssaj2003.1629.

Donohue, K. (2002) ‘Germination Timing Influences Natural Selection on Life-History Characters in Arabidopsis thaliana’, *Ecology*, 83(4), pp. 1006–1016.

Donohue, K. (2005) ‘Seeds and seasons: interpreting germination timing in the field’, *Seed Science Research*, 15(3), pp. 175–187. doi: 10.1079/ssr2005208.

Donohue, K. *et al.* (2010) ‘Germination, postgermination adaptation, and species ecological ranges’, *Annual Review of Ecology, Evolution, and Systematics*, 41, pp. 293–319. doi: 10.1146/annurev-ecolsys-102209-144715.

Drescher, M. (2014) ‘Snow cover manipulations and passive warming affect post-winter seed germination: A case study of three cold-temperate tree species’, *Climate Research*, 60(3), pp. 175–186. doi: 10.3354/cr01237.

Drescher, M. and Thomas, S. C. (2013) ‘Snow cover manipulations alter survival of early life stages of cold-temperate tree species’, *Oikos*, 122(4), pp. 541–554. doi: 10.1111/j.1600-0706.2012.20642.x.

Fernández-Pascual, E. *et al.* (2017) ‘Comparative seed germination traits in alpine and subalpine grasslands: higher elevations are associated with warmer germination temperatures’, *Plant Biology*, 19(1), pp. 32–40. doi: 10.1111/plb.12472.

Fernández-Pascual, E. *et al.* (2021) ‘The seed germination spectrum of alpine plants: a global meta-analysis’, *New Phytologist*, 229(6), pp. 3573–3586. doi: 10.1111/nph.17086.

Frei, P. *et al.* (2018) ‘Future snowfall in the Alps: Projections based on the EURO-CORDEX regional climate models’, *Cryosphere*, 12(1), pp. 1–24. doi: 10.5194/tc-12-1-2018.

Gerdol, R. *et al.* (2013) ‘Advanced snowmelt affects vegetative growth and sexual reproduction of Vaccinium myrtillus in a sub-alpine heath’, *Journal of Vegetation Science*, 24(3), pp. 569–579. doi: 10.1111/j.1654-1103.2012.01472.x.

Giménez-Benavides, L. *et al.* (2018) ‘How does climate change affect regeneration of Mediterranean high-mountain plants? An integration and synthesis of current knowledge’, *Plant Biology*, 20, pp. 50–62. doi: 10.1111/plb.12643.

Giménez-Benavides, L., Escudero, A. and Pérez-García, F. (2005) ‘Seed germination of high mountain Mediterranean species: Altitudinal, interpopulation and interannual variability’, *Ecological Research*, 20(4), pp. 433–444. doi: 10.1007/s11284-005-0059-4.

Graham, E. *et al.* (2012) ‘Fine-scale patterns of soil and plant surface temperatures in an alpine fellfield habitat, white mountains, California’, *Arctic, Antarctic, and Alpine Research*, 44(3), pp. 288–295. doi: 10.1657/1938-4246-44.3.288.

Graham, J. K., Smith, M. L. and Simons, A. M. (2014) ‘Experimental evolution of bet hedging under manipulated environmental uncertainty in Neurospora Crassa’, *Proceedings of the Royal Society B: Biological Sciences*, 281(1787). doi: 10.1098/rspb.2014.0706.

Gremer, J. R. *et al.* (2020) ‘Variation in the seasonal germination niche across an elevational gradient: the role of germination cueing in current and future climates’, *American Journal of Botany*, 107(2), pp. 350–363. doi: 10.1002/ajb2.1425.

Gremer, J. R. and Venable, D. L. (2014) ‘Bet hedging in desert winter annual plants: Optimal germination strategies in a variable environment’, *Ecology Letters*, 17(3), pp. 380–387. doi: 10.1111/ele.12241.

Hadfield, J. D. (2010) ‘MCMCglmm: MCMC Methods for Multi-Response GLMMs in R’, *Journal of Statistical Software*, 33(2), pp. 1–22. Available at: http://www.jstatsoft.org/.

Hopp, R. J. (1974) ‘Plant Phenology Observation Networks’, in Lieth, H. (ed.) *Phenology and Seasonality Modeling. Ecological Studies, vol 8.* Berlin, Heidelberg: Springer. doi: https://doi.org/10.1007/978-3-642-51863-8\_3.

Hoyle, G. L. *et al.* (2015) ‘Seed germination strategies: An evolutionary trajectory independent of vegetative functional traits’, *Frontiers in Plant Science*, 6(OCTOBER), pp. 1–13. doi: 10.3389/fpls.2015.00731.

Huang, Z. *et al.* (2016) ‘The contribution of germination functional traits to population dynamics of a desert plant community’, *Ecology*, 97(1), pp. 250–261. doi: 10.1890/15-0744.1.

Jiménez-Alfaro, B. *et al.* (2021) ‘Checklist of the vascular plants of the Cantabrian Mountains’, *Mediterranean Botany*, 42, pp. 1–60. doi: 10.5209/MBOT.74570.

Jiménez-Alfaro, B. *et al.* (2024) ‘Journal of Vegetation Science Spatiotemporal patterns of microclimatic buffering in relict alpine communities’, *Journal of Vegeta*, (July 2023). doi: 10.1111/jvs.13242.

Jin, Y. and Qian, H. (2019) ‘V.PhyloMaker: an R package that can generate very large phylogenies for vascular plants’, *Ecography*, 42(8), pp. 1353–1359. doi: 10.1111/ecog.04434.

Karger, D. N. *et al.* (2017) ‘Climatologies at high resolution for the earth’s land surface areas’, *Scientific Data*, 4, pp. 1–20. doi: 10.1038/sdata.2017.122.

Keck, F. *et al.* (2016) ‘phylosignal: an R package to measure, test, and explore the phylogenetic signal’, *Ecology and Evolution*, 6(9), pp. 2774–2780. doi: 10.1002/ece3.2051.

Kimball, S. *et al.* (2011) ‘Differences in the timing of germination and reproduction relate to growth physiology and population dynamics of sonoran desert winter annuals’, *American Journal of Botany*, 98(11), pp. 1773–1781. doi: 10.3732/ajb.1100034.

Körner, C. (2021) *Alpine Plant Life*. 3rd edn. Edited by Springer Nature Switzerland AG 2021. Springer Cham. doi: 10.1007/978-3-030-59538-8.

Levine, J. M., Mceachern, A. K. and Cowan, C. (2011) ‘Seasonal timing of first rain storms affects rare plant population dynamics’, *Ecology*, 92(12), pp. 2236–2247.

M. Pagel (1999) ‘Inferring the historical patterns of biological evolution’, *Nature*, 401(October), pp. 877–884.

Mattana, E. *et al.* (2022) ‘Climate change and plant regeneration from seeds in Mediterranean regions of the Northern Hemisphere’, in *Plant Regeneration from Seeds A Global Warming Perspective*. Academic Press, pp. 101–114.

Mondoni, A. *et al.* (2009) ‘Germination requirements of the alpine endemic Silene elisabethae Jan: Effects of cold stratification, light and GA3’, *Seed Science and Technology*, 37(1), pp. 79–87. doi: 10.15258/sst.2009.37.1.10.

Mondoni, A. *et al.* (2012) ‘Climate warming could shift the timing of seed germination in alpine plants’, *Annals of Botany*, 110(1), pp. 155–164. doi: 10.1093/aob/mcs097.

Mondoni, A. *et al.* (2015) ‘Climate warming could increase recruitment success in glacier foreland plants’, *Annals of Botany*, 116(6), pp. 907–916. doi: 10.1093/aob/mcv101.

Mondoni, A., Jiménez-Alfaro, B. and Cavieres, L. A. (2022) ‘Effect of climate change on plant regeneration from seeds in the arctic and alpine biome’, in *Plant Regeneration from Seeds*. Academic Press.

Poschlod, P. *et al.* (2013) *Seed Ecology and Assembly Rules in Plant Communities*, *Vegetation Ecology: Second Edition*. doi: 10.1002/9781118452592.ch6.

R Hackathon et al. (2020) ‘phylobase: Base Package for Phylogenetic Structures and Comparative Data’. Available at: https://cran.r-project.org/package=phylobase.

Rosbakh, S. *et al.* (2022) ‘Alpine plant communities differ in their seed germination requirements along a snowmelt gradient in the Caucasus’, *Alpine Botany*, 132(2), pp. 223–232. doi: 10.1007/s00035-022-00286-x.

Rosbakh, S. and Poschlod, P. (2015) ‘Initial temperature of seed germination as related to species occurrence along a temperature gradient’, *Functional Ecology*, 29(1), pp. 5–14. doi: 10.1111/1365-2435.12304.

Scherrer, D. and Körner, C. (2011) ‘Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming’, *Journal of Biogeography*, 38, pp. 406–416. doi: https://doi.org/10.1111/j.1365-2699.2010.02407.x.

Schwienbacher, E. *et al.* (2011) ‘Seed dormancy in alpine species’, *Flora: Morphology, Distribution, Functional Ecology of Plants*, 206(10), pp. 845–856. doi: 10.1016/j.flora.2011.05.001.

Schwienbacher, E. *et al.* (2012) ‘Correspondence of seed traits with niche position in glacier foreland succession’, *Plant Ecology*, 213(3), pp. 371–382. doi: 10.1007/s11258-011-9981-4.

Schwienbacher, E., Marcante, S. and Erschbamer, B. (2010) ‘Alpine species seed longevity in the soil in relation to seed size and shape - A 5-year burial experiment in the Central Alps’, *Flora: Morphology, Distribution, Functional Ecology of Plants*, 205(1), pp. 19–25. doi: 10.1016/j.flora.2008.10.007.

Scranton, K. and Amarasekare, P. (2017) ‘Predicting phenological shifts in a changing climate’, *Proceedings of the National Academy of Sciences of the United States of America*, 114(50), pp. 13212–13217. doi: 10.1073/pnas.1711221114.

Segrestin, J. *et al.* (2018) ‘When is the best time to flower and disperse? A comparative analysis of plant reproductive phenology in the Mediterranean’, *Functional Ecology*, 32(7), pp. 1770–1783. doi: 10.1111/1365-2435.13098.

Segrestin, J., Navas, M. L. and Garnier, E. (2020) ‘Reproductive phenology as a dimension of the phenotypic space in 139 plant species from the Mediterranean’, *New Phytologist*, 225(2), pp. 740–753. doi: 10.1111/nph.16165.

Shimono, Y. and Kudo, G. (2005) ‘Comparisons of germination traits of alpine plants between fellfield and snowbed habitats’, *Ecological Research*, 20(2), pp. 189–197. doi: 10.1007/s11284-004-0031-8.

Simons, A. M. (2011) ‘Modes of response to environmental change and the elusive empirical evidence for bet hedging’, *Proceedings of the Royal Society B: Biological Sciences*, 278(1712), pp. 1601–1609. doi: 10.1098/rspb.2011.0176.

Thomson, Di. M., King, R. A. and Schultz, E. L. (2017) ‘Between invaders and a risky place: Exotic grasses alter demographic tradeoffs of native forb germination timing’, *Ecosphere*, 8(10). doi: 10.1002/ecs2.1987.

Tudela-Isanta, M., Fernández-Pascual, E., *et al.* (2018) ‘Habitat-related seed germination traits in alpine habitats’, *Ecology and Evolution*, 8(1), pp. 150–161. doi: 10.1002/ece3.3539.

Tudela-Isanta, M., Ladouceur, E., *et al.* (2018) ‘The seed germination niche limits the distribution of some plant species in calcareous or siliceous alpine bedrocks’, *Alpine Botany*, 128(1), pp. 83–95. doi: 10.1007/s00035-018-0199-0.

Verdú, A. and Traveset, A. (2005) ‘EARLY EMERGENCE ENHANCES PLANT FITNESS: A PHYLOGENETICALLY CONTROLLED META-ANALYSIS’, *Ecology*, 86(6), pp. 1385–1394. doi: 10.1890/04-1647.

Wagner, I. and Simons, A. M. (2009) ‘Divergence in Germination Traits among Arctic and Alpinepopulations of Koenigia islandica: Light Requirements’, *Plant Ecology*, 204(1), pp. 145–153. doi: 10.1007/sl 1258-009-9578-3.

Walck, J. L. *et al.* (2011) ‘Climate change and plant regeneration from seed’, *Global Change Biology*, 17(6), pp. 2145–2161. doi: 10.1111/j.1365-2486.2010.02368.x.

Zhang, Y. *et al.* (2005) ‘Soil temperature in Canada during the twentieth century: Complex responses to atmospheric climate change’, *Journal of Geophysical Research D: Atmospheres*, 110(3), pp. 1–15. doi: 10.1029/2004JD004910.

**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 T mean > 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. |

Gráfico

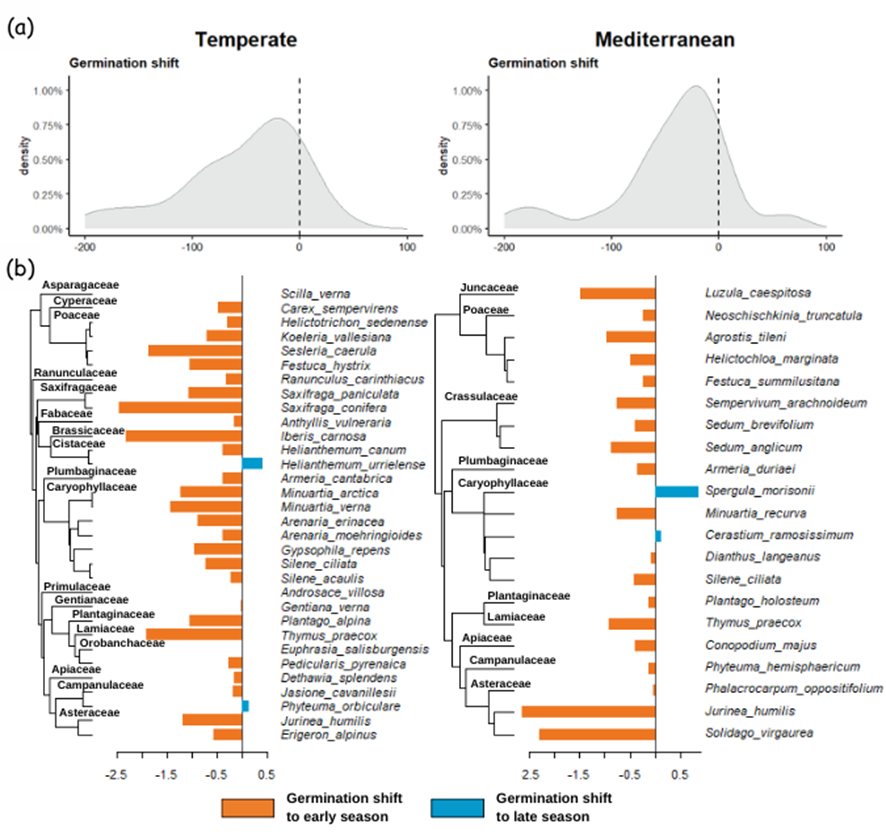
Descripción generada automáticamente

**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 conditions in laboratory incubators. Both incubators were configured based on real field data showed in C.

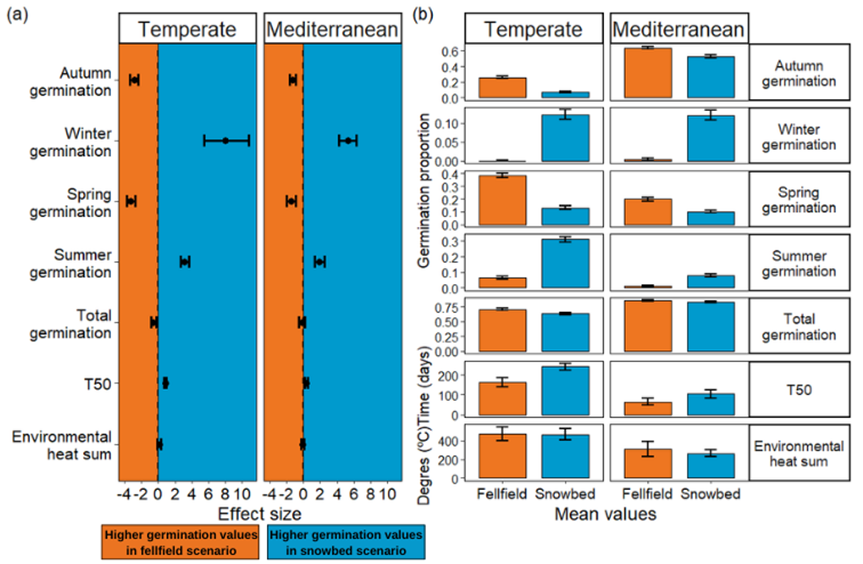
Imagen que contiene Diagrama

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 and blue curves represent germination proportion in the fellfield and snowbed conditions, respectively. Flat areas represent cold period when no germination scores were done. Density plots within each system represents the calculated area between individual species cumulative germination curves. At the bottom horizontal bars represent the phenology periods calculated. (b) Cumulative germination curves for each individual species in the fellfield incubator from both systems. (c) Show cumulative germination curves for each individual species in the snowbed incubator from both systems.



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



**Fig. 4** Germination phenology traits responses for both incubators in each system. (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 conditions (blue background), while dots on the left side of the zero-line (negative) mean higher values in the fellfield conditions (orange background). (b) Mean germination values for the seven traits at each conditions for both systems. 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.

Diagrama, PowerPoint

Descripción generada automáticamente

**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 (early season vs late season).