

A COMPARATIVE GERMINATION STUDY AMONG DIFFERENT ALPINE HABITATS.

Thesis dissertation



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A comparative germination study among different alpine habitats

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Certification

I, Maria Tudela Isanta, declare that this thesis, submitted in partial fulfilment of the requirements for the award Doctor of Philosophy, in the Department of Earth and Environmental Sciences, University of Pavia, is wholly my own work unless otherwise referenced or acknowledged. This document has not been submitted for qualifications at any other academic institution.

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Statement of contribution

The three data chapters (i.e. Chapter 2, 3 and 5) presented in this thesis have been prepared as manuscripts in collaboration with my supervisor Andrea Mondoni. These chapters have been written as the following journal articles:

- Chapter 2. Habitat-related seed germination behavior in alpine habitats.
- Chapter 3. Germination niche limiting species distribution in alpine bedrocks.
- Chapter 4. Thermal germination models suggest distinct timing of emergence in alpine plants inhabiting the same growing site.

Statement of style

This thesis has been prepared in journal article compilation style format. With the exception of the Chapter 1 (General Introduction) and Chapter 5 (General discussion), each chapter has been written with the aim of publication in an ecological journal. Because of this there is some overlap between chapters, particularly in relation to the description of the habitat type.

A la padrina i a la iaia,

When the wind calls, you know, that somewhere in the mountains, it has found the answers that you were looking for. The pull of the horizon overcomes the inertia of reason...And you just have to go.

Vikram Oberoi

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Abstract

A comparative germination study among different alpine habitats.

If species inhabit in a specific habitat two conditions should be befallen: first seeds must be present through dispersal or in the soil seed bank and secondly, conditions for germination must occurred. If suitable germination niche is not met, germination will not occur and, consequently, species will not be present. In an alpine context, the large species and microhabitats pool found have resulted in a variety of germination and dormancy patterns, which make it difficult to define a common alpine germination niche or behaviour. Therefore, to better understand germination ecology in alpine environment and their functional role in filtering the regional species pools into local communities a habitat-related germination study is needed. A habitat-related approach will contribute to a better comprehension of which are the key factors affecting germination in alpine habitats and define the different alpine germination niches. For this reason, during this research period, I described and compare the type and level of seed dormancy and assessed species' germination strategies of 53 alpine species growing in two different habitats. Subsequently I defined the germination niche of seven pairs of closely related species inhabiting in these habitats. Finally, I predict species germination timing in the field using thermalmodels. To this end seeds were collected from two Sites of Community Interest (SCI) "Passo dello Stelvio (46° 32'N, 10° 25'E)" and "Val Viola, Dosde (46° 24'N, 10°12E)", located in the Alps of Lombardy (Sondrio, Northern Italy). These SCI belong to two habitats types defined by the European Habitat Directive Natura 2000 classification: Habitat #6230- Nardus-rich species grasslands" or "siliceous habitat"; and Habitat #6170- Alpine calcareous grasslands' or "calcareous habitat".

Firstly, as mentioned, seeds of 53 species growing in alpine siliceous and calcareous habitats were exposed to different temperatures (i.e. 25/15°C; 15/5°C) and treatments (i.e. 0,3 or 5 months of cold stratification and seeds were sown with gibberellic acid) under controlled laboratory conditions. Germination strategies in each habitat were identified by clustering and species' germinability and dormancy were compared between habitat (calcareous and siliceous), among microhabitat (grasslands, heaths, rocky and species with no specific microhabitats) and between chorology type (arctic-alpine and continental) using phylogenetic least squares correlations (PGLS). Results showed that calcareous and siliceous species showed different germination strategies, with a slow, mostly overwinter germination on calcareous species', and with fast and undifferentiated timing germination for siliceous ones. Moreover, specialized regenerative strategies were observed among microhabitats with species mostly occurring in heaths showing high overwinter germination. In conclusion, alpine species use different germination strategies depending on habitat provenance, species' main microhabitat and chorotype.

Next in line, we defined the vegetative and germination traits of 14 closely related species growing in the two target alpine habitats- calcareous and siliceous. Germination niche derived from laboratory-controlled test, including cardinal temperatures for germination, base water-potential and pH range of germination. Meanwhile, vegetative traits were defined using species' leaf area, specific leaf area and leaf dry matter content, by querying TRY-database. Habitat dissimilarities in both vegetative and germination traits were assessed. Results showed some germination traits dissimilarities according to habitat provenience. Conversely, other germination traits and vegetative traits were similar in both habitats. The high vegetative traits convergence hints at small differentiations in adult plants between habitats. However, the separate habitat clusters when using germination traits and the significant differences detected when assessing habitat dissimilarities, suggest that species occurrence in the two habitats may be limited at the earliest stages of plant development, particularly by germination. Conversely, the similar germination traits observed between habitats may indicate common plant adaptations to alpine environment that should prevent germination in late summer, such as narrow temperature range at the time of seed dispersal and high optimal temperature for germination.

Finally, germination timing of three alpine plants showing different germination strategies was estimated using thermal models. Base temperature (T_b) and thermal time (θ_{50}) for germination was estimated after different periods of cold stratification. Subsequently, temperature data from the soil was combined to estimate species soil heat sum and consequently, species' germination timing. Results showed species were dormant when dispersal though different responses to cold stratification were identified. T_b and θ_{50} differed among species, consequently soil heat models predicted different species' germination timing. The different dormancy types detected here indicate that dormancy may play a significant role in controlling germination, always awarding risk-averse strategies to escape winter. Thermal models contributed to understand germination timing in the study species demonstrating that germination timing is avoided at time of dispersal. However, the differentiated germination timings reduce probability to overlap species' germination niche, thus having different timings species do not compete for the same resources.

In conclusion, this thesis describes germination ecology on alpine habitats and identify the main factors affecting species' specific germination behavior. Special attention was putted on the possible functional role of germination and dormancy on driving species distribution and community assembly. I highlighted the needed of doing more research on regeneration processes *in situ* and in the laboratory to find out standardized measurements and protocols to measure germination. Finally, all outputs reached here and future outputs that new research will bring hint at important implication in developing good practices for restoration and conservation activities in alpine habitats and contribute to a better understanding of the response of alpine plants in the regeneration from seeds due to global warming.

List of publications

Papers in International Journals with Peer-Review

Tudela-Isanta, M, Fernández-Pascual E, Wijayasinghe M, Orsenigo S, Pritchard HW, Rossi G, Mondoni A. 2017. Habitat-related seed germination traits in alpine habitats. *Ecology and Evolution*. In press.

Tudela-Isanta, M, Ladouceur ER, Wijayasinghe M, Pritchard HW, Mondoni A. 2017. Seed germination niche contributes to limit some plant species distributions to calcareous or siliceous alpine bedrocks. *Alpine Botany* (Accepted)

Communications in International Congresses as First Author

Tudela-Isanta, M, Wijayasinghe M, Rossi G, Mondoni A. 2015. Patterns of seed germination within alpine grasslands of different provenience and habitats. III International Plant Science Conference (IPSC), September 2015, Pavia (Italy).

Tudela-Isanta, M, Orsenigo S, Rossi G, Wijayasinghe M, Mondoni A. 2016. Is germination of alpine species modeled by habitat provenience? Seed Ecology Congress, August 2016, Minas Gerai (Brasil).

Tudela-Isanta, M, Wijayasinghe M, Pritchard HW, Mondoni A. 2017. Seed germination traits in alpine grasslands play a part in species' assembly. XIX International Botanical Congress, July 2017, Shenzhen (China).

Tudela-Isanta, M, Wijayasinghe M, Pritchard HW, Mondoni A. 2017. Do germination traits limit species' distribution in alpine habitats? Seed Quality of Native Species-Ecology, Production and Policy, September 2017, London (UK).

Communications in International Congresses as Co-author

Blandino C, Frischie S, López del Egidio L, **Tudela-Isanta, M**. 2015. Dormancy and germination: their potential impact on seed production and plant establishment. XXVII International Congress for Conservation Biology, August 2015, Montpellier (France)

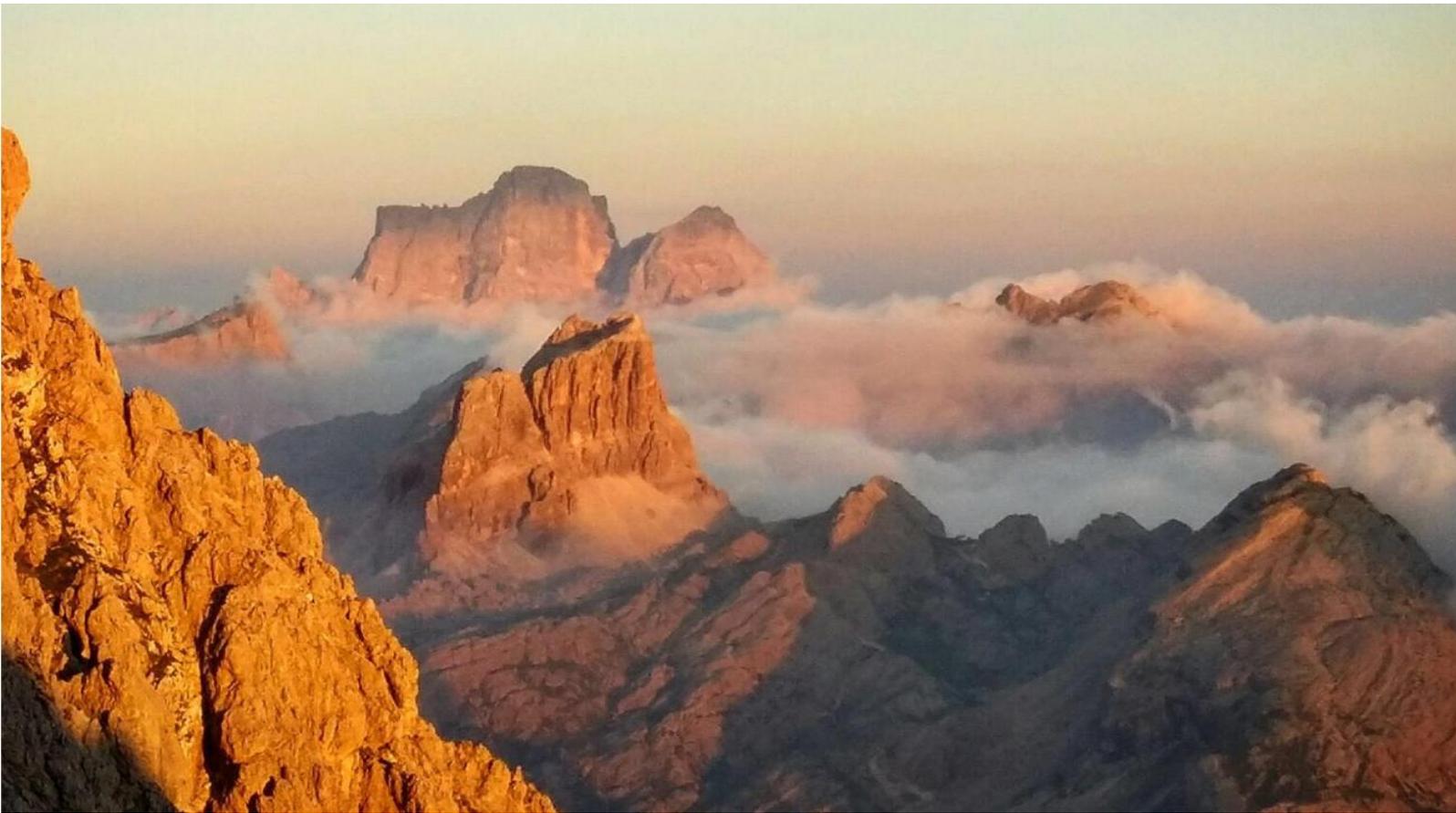
Mondoni A, **Tudela-Isanta, M.** 2017. Seed germination and alpine climate change. XIX International Botanical Congress, July 2017, Shenzhen (China).

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Chapter 1

Introduction: The role of germination in alpine habitats



1.1 General introduction: “Why do species occur where they occur?”

“Why do species occur where they occur?”, during centuries plant ecologists have been investigating factors driving species’ occurrence. The answer to this question will allow to develop predicting rules for understanding species behavior in front of biotic and abiotic disturbances, thereby understanding, for example, species risk of extinction because of climate change, developing adequate practices for restoration and conservation aims. To this end, many theories including the view of community as a super-organism (Clements 1916) or the individualistic concept of species (Gleason 1926) attempt to understand the main rules behind vegetation patterns; however, there is still little consensus of how species assemble in the natural environment (Austin, 2013). Nevertheless, it has long been acknowledged that environmental pressures limit plant populations recruitment (e.g.(Jump *et al.* 2009; Amano *et al.* 2014) which link with the statements of species niches theory. Species niches are “the multidimensional space of environmental factors in which the rate of population increase positively”(Hutchinson 1957). Therefore, plant community may be modeled by species partitioning a resource, for example competing for water. Alpine habitats, which are characterized by steeper environmental gradients provide an ideal context to study the effect of the different abiotic factors such as water availability, temperature, nutrients or pH on plant distribution, in particular, in plant germination niche. Furthermore, these ecosystems are known to be rich in endemism and plant diversity (Körner 2003). For these reasons and for their huge beauty, alpine bioregion was chosen as a study area for my thesis.

Species niche differentiation can be defined by the traits of the species inhabiting in a particular habitat (Rosenzweig 1987; Chase and Leibold 2003). Functional traits provide taxonomic independent tools instead of species identity to generalize the structure and function of plant communities (McGill *et al.* 2006). Hence, traits could be a step toward unifying community ecology theory (Funk *et al.* 2016). Furthermore, numerous studies demonstrate that interspecific functional traits differences are linked to environmental gradients (see Wright *et al.* 2005; Rosbakh and Poschlod 2016) and/or demographic responses (Poorter and Markesteijn 2008). Therefore, species with physiological limitations to face abiotic conditions will be excluded from the community. Convergence on traits within a community hint selective forces driving the evolution of specific strategies. For example, species living in drier ecosystem show lower specific leaf area (SLA) than the observed mean in species regional pool (Cornwell and Ackerly 2009). Indeed, big efforts have been done incorporating traits into statistical models

for projecting species distributions under the new expected climate scenarios (see Elith and Leathwick 2009) or linking traits to ecosystem function (Suding *et al.* 2008). Despite progress, the appropriate trait selection (Laughlin 2014) and the existence of a standardized methodology for trait measurement are challenges that still need to be faced.

During the plant life cycle species may have different requirements, for instance, some plants need periods of vernalization to promote flowering (i.e. *Arabidopsis thaliana* (Sung and Amasino 2004)) or cold stratification for germination (i.e. *Arenaria grandiflora* (Fernández-Pascualet al. 2017)). Thus, the environmental forces filtering species niche and consequently the informative plant traits may be different in each plant stage. Accordingly, Grubb (1977) defined plant niche with four components: habitat, the life-form, the phenological and the regeneration niche. Each of these niches is defined by different parameters; reproduction niche, for instance, is mainly limited by physical and chemical requirements for species' survival and reproduction. Meanwhile, the life-form niche is mainly explained by species' physiological or ecological parameters (e.g. growth rate). The regeneration processes are considered key determinants of population growth and abundance (Grubb 1977; Harsch *et al.* 2014; Huang *et al.* 2016). However, they are rarely considered when inferring species' assembly rules (Laughlin 2014). Regeneration is understood by Grubb (1977) as 'the process whether a mature individual of the population is replaced by a new individual of the population'. Regeneration niche, then, includes all processes which ensure success in viable seed production, seed dispersal, seed germination and seedling survival.

Moreover, if species inhabits in a specific habitat, first seeds must be present through dispersal or in the soil seed bank. Dispersal events allow plant species to recolonize unoccupied sites or colonized new successful habitats (Poschlod *et al.* 2013) while soil seeds bank ensure population recruitment when unfavorable climatic conditions occurred during consecutive years (Ooi 2012). Therefore, seed dispersal and seed bank persistence play important roles in species abundance and occurrence. Nevertheless, the presence of seeds is essential, the appropriate germination conditions must also occur (Donohue *et al.* 2010). If the suitable germination niche is not met, germination will not occur and, consequently, species will not be present. Indeed, seed germination is extremely regulated by the environment (Fenner and Thompson 2005) and even, the environmental conditions experienced by mother plants during flowering and dispersal time, will in turn influence the offspring germinability (Luzuriaga *et al.* 2006) and dormancy state (Carta *et al.* 2016). When and where seeds germinate determine not only the season in which seedling will grow but also the likelihood of seedling recruitment

(Salazar *et al.* 2011). Consequently, some plants develop germination controlling mechanisms, in which specific environmental conditions or sequences of environmental events must occur to elicit germination (Baskin and Baskin 2014).

One of these mechanisms is dormancy. A seed is dormant when “*fail to germinate even though the environmental conditions including water, temperature, light and gases are favorable for germination*” (Baskin and Baskin 2014). According with Baskin and Baskin (2014) classification system of dormancy a seed can be: non-dormant (ND) then it will germinate, morphologically dormant (MD) (i.e. seed has small or underdeveloped embryo when dispersal); physical dormant (PY) (i.e. seeds which have water impermeable seed coats or endocarp, then water is prevented from reaching the embryo); physiological dormant (PD) (i.e. seeds has a physiological block that prevent radicle emergence), or a combination of dormancy including PY+PD or MD+PD (MPD). Dormancy seems to play key roles in species assembly on a global scale, for instance in temperate and arctic biomes there is higher proportion of species dispersing dormant seeds than in subtropical and tropical zones (Baskin and Baskin 2014). Indeed, the environmental requirements to release dormancy are related with the habitat unsuitable conditions for growing. For example, arctic species with physiological dormancy needs long periods of cold stratification to release dormancy (Cavieres and Arroyo 2000), meanwhile many species inhabiting in arid habitats, dormancy is released during summer when they are exposed to high temperatures (Ooi *et al.* 2009).

Germination, an irreversible process, is also controlled by a series of environmental cues including temperature, moisture and often light (Bewley *et al.* 2013). Firstly, light perception for a seed means that they are close to or in the soil surface, hence the likelihood of seedling establishment is high. Indeed, smaller-seeded species from temperate herbaceous habitats showed stronger light requirement for germination than larger-seeded species (Milberg *et al.* 2000), because small seeds become easily buried in the soil. Secondly, temperature plays an important role for seed germination (in addition to induces or breaks dormancy). Indeed, temperature-response to germination was attributed to species provenance (Mondoni, *unpublished results*) or correlated with elevation gradients (Fernández-Pascual *et al.* 2017), indicating a strong selective pressure of the environment. Finally, seed imbibition is an essential prerequisite for germination (Preston *et al.* 2009); the rate water enters to the seeds depend on seed coats' permeability and the water availability in the substrate (Bradford 1995). Each species requires a critical amount of water for germination (Hunter and Erickson 1952) and it is highly species-specific.

The importance of water and temperature enhancing germination bring to develop mathematical models that aim to describe germination patterns in response to these factors. These models assume that optimal water and temperature values at which germination is maximum exists. Below and above this optimum, germination decreases progressively until stops defining base (base temperature or T_b and minimal water potential or Ψ_m) and ceiling (maximum temperature or T_c) thresholds. These thresholds represent the lowest (T_b and Ψ_m) or the maximum (T_c) temperature or water requirement for germination. Germination rate between the base and the optimal threshold; and between the optimal and the maximal threshold for a given fraction of the set of seeds are defined as a linear function of temperature (García-Huidobro *et al.* 1982) or water potential (Gummerson 1986) with a slope (1/hydro or thermal constant). Therefore, germination timing can be described as temperature or water potential in excess of the base threshold; when a certain amount of °C-day or MPa-day have been accumulated seeds will germinate. Thermal and hydromodels are useful tools often used in agriculture to predict time of weed emergence (Batlla *et al.* 2009). Consequently, appropriate land management techniques can be designed and to select the more suitable month for applying herbicide. Moreover, these models provide some informative and standardized germination traits (e.g. T_b , T_c , Ψ_m) that can be easily comparable and incorporate in the community ecology models, filling the standardized germination traits gap.

1.2 Germination and dormancy in an alpine context.

In the alpine context, species germination ecology is complex. The light had, in general, a positive effect on enhancing seed germination (Bu *et al.* 2016), while the role of temperature is not clear. For example, Walder and Erschbamer (2015) showed that species from alpine habitats resulted in a higher optimal temperature for germination than their congeneric growing below the treeline. However, despite higher temperature requirement for germination have been attributed to alpine species (Billings 1974), germination at cold stratification temperatures have also been described (Schwienbacher *et al.* 2011; Hoyle *et al.* 2015; Fernández-Pascual *et al.* 2017). Water restriction generally decreased germination on alpine plants (Orsenigo *et al.* 2015; Walder and Erschbamer 2015), but little is known about the effect of drought on these species. Indeed, germination of alpine species is thought to be in late spring, after the snow-melt (Körner 2003; Schwienbacher *et al.* 2011), when water is not a limiting factor. To ensure germination when winter has passed, seeds are usually PD at dispersal (Schwienbacher *et al.* 2011; Sommerville *et al.* 2013) and become non-dormant after a period of cold stratification.

Nevertheless, non-dormant seeds or species with stagger germination are also typical from the alpine habitat (Schwienbacher *et al.* 2011; Hoyle *et al.* 2015). The large species and microhabitat pool found in alpine habitats have resulted in a variety of germination and dormancy patterns, which make it difficult to define a common alpine germination behaviour. For example, Schwienbacher *et al.* (2012) highlighted that alpine pioneer species germinate better at colder temperatures than later successional species. However, no habitat related germination strategies were identified when comparing fellfield and snowbed habitats (Shimono and Kudo 2005). Dormancy patterns in alpine seeds are also influenced by species life history, with arctic-alpine species being more dormant than alpine-Mediterranean endemics (Giménez-Benavides *et al.* 2007). Moreover, altitudinal effect on species' germinability have also been reported, with populations from high altitude germinating faster and more (Vera 1997) than those from lower altitude. It follows that, specific regenerative strategies have evolved in response to the different local environmental pressure hinting the key role of germination in plant life.

Despite germination traits are rarely considered in plant community ecology studies (Jiménez-Alfaro *et al.* 2016; Larson and Funk 2016), recently, they are receiving an increasing attention (De Vitis *et al.* 2014; Valladares *et al.* 2014; Fernández-Pascual *et al.* 2015; Mackenzie *et al.* 2016; Carta *et al.* 2017) and regeneration from seeds is necessary for the maintenance of alpine plant communities. Therefore, to better understand germination ecology in alpine environment and their functional role in filtering the regional species pools into local communities (Jiménez-Alfaro *et al.* 2016) a habitat-related germination study is needed. A habitat-related approach will contribute to a better comprehension of which are the key factors affecting germination in alpine habitats and define the different alpine germination niches. This knowledge is urgently needed to face the risk of biodiversity loss due to climate warming in these environments (Parolo and Rossi 2008; Orsenigo *et al.* 2014), selecting appropriate regeneration treatments (Merritt *et al.* 2011) and species for effective restoration practices at a local and global scale. In this thesis, I aim to describe and compare the type and level of seed dormancy and to assess species' germination strategies of 53 alpine species growing in two different habitats. Subsequently I will define the germination niche of each species inhabiting in these habitats. Finally, germination time of three target alpine species inhabiting in alpine habitats will be predicted using thermal models.

1.3 Study site

Seeds were collected from Site of Community Interest (SCI) “Passo dello Stelvio (46° 32’N, 10° 25’E)”, located in the Alps of Lombardy (Sondrio, Northern Italy). This SCI belongs to two habitats types defined by the European Habitat Directive Natura 2000 classification: Habitat #6230- Nardus-rich species grasslands’ (referred to as “siliceous habitat” throughout); and Habitat #6170- Alpine calcareous grasslands’ (referred to as “calcareous habitat” throughout) (European commission, 2007). These habitats were chosen because they represent two main vegetation types of the European high alpine zone, and are habitats of conservation concern. Despite these two habitats have similar climatic conditions (in terms of mean annual temperature, the total amount of precipitation/year and so on), they have completely different plant communities and soil properties (Nagy and Grabherr 2009). Habitat #6230 occurs in nutrient-poor soils with acidic pH; Al^+ and H^+ are frequent and available cations, formed on various types of siliceous rocks (mainly crystalline slides and granite, and volcanic rock). Its frequent plant species include *Nardus stricta*, *Carex curvula* and *Arnica montana* (Gennai *et al.* 2014). Meanwhile, habitat #6170 is characterized by nutrient-rich soils and alkaline pH, formed on calcareous bedrocks; Ca^{2+} cations are present instead of Al^+ or H^+ . Furthermore, calcareous bedrocks are normally drier (Körner 2003) because the soil holding capacity is lower in these soils, compared to that of siliceous soil. Its typical plant species include *Sesleria caerulea*, *Dryas octopetala* and *Phyteuma orbiculare* (European Commission 2007).

1.4 Aims and structure of the work

This research is part of NASSTEC (The NATive Seed Source Science, Technology and Conservation Initial Training Network). Seven main partners, including seed companies (i.e. Semillas Silvestres-Spain; Scotia Seeds-Scotland; Syngenta-Netherlands), research institutions (i.e. KEW-United Kingdom; James Hutton Institute-Scotland; MuSe-Trento) and one university (University of Pavia), are a functioning network. The main aim of this project is to promote the native seed production and use in grassland restoration building industrial capacity in local companies. While forming and training 11 Early Stage Researchers and 1 Experienced Researcher in native seed science, conservation and use, to increase the impact of environmental mitigation and adaptation projects. NASSTEC is supported by the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme FP7/2007-2013/ under REA grant agreement n°607785.

This thesis is comprised of four chapters, including a general introduction (Chapter 1), two data chapters (Chapters 2 and 3) and a general discussion (Chapter 4). In Chapter 2, I aim to clarify germination patterns in alpine species and add further insights into germination functional significance role of in community ecology. To this end, I assessed germination phenology and dormancy of 53 species inhabiting in calcareous (#6170) and siliceous (#6230) habitats.

In Chapter 3, I am to identify if germination niche may be limiting species' distribution in alpine habitats. To this end, germination traits (i.e. including Cardinal temperatures, minimum water potential and pH germination range) and vegetative traits (i.e. including Specific leaf area, Leaf area and leaf dry matter content) of seven closely related species (i.e. 6 co-generic and 1 co-family species) inhabiting in in calcareous (#6170) and siliceous (#6230) habitats were defined and compared.

In Chapter 4, I aim to modelized germination timing in alpine habitats. The minimal temperature for germination of three target species inhabiting in siliceous habitats (#6230) has been defined after different cold stratification periods. Then, temperature soil data from the species-growing site were used to calculate species soil heat sum and consequently species' germination timing in the field were predicted.

Finally, in Chapter 5, I summarize the main outcomes found here and discuss the implications of these findings in ecology, with a special mention of the role of germination traits in limiting species assembly.

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Chapter 2

Habitat-related seed germination behavior in alpine habitats.



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Abstract

Understanding the key aspects of plant regeneration from seeds is crucial in assessing species assembly to their habitats. However, the regenerative traits of seed dormancy and germination are underrepresented in this context. In the alpine zone, the large species and microhabitat diversity provide an ideal context to assess habitat-related regenerative strategies. To this end, seeds of 53 species growing in alpine siliceous and calcareous habitats (6230 and 6170 of EU Directive 92/43, respectively) were exposed to different temperature treatments under controlled laboratory conditions. Germination strategies in each habitat were identified by clustering with k-means. Then, phylogenetic least squares correlations (PGLS) were fitted to assess germination and dormancy differences between species' main habitat (calcareous and siliceous), microhabitat (grasslands, heaths, rocky and species with no specific microhabitats) and chorology (arctic-alpine and continental). Calcareous and siliceous grasslands significantly differ in their germination behaviour with a slow, mostly overwinter germination and high germination under all conditions, respectively. Species with high overwinter germination occurs mostly in heaths and have an artic-alpine distribution. Meanwhile, species with low or high germinability in general inhabiting in grasslands or having no specific microhabitat, respectively. Alpine species use different germination strategies depending on habitat provenance, species' main microhabitat and chorotype. Such differences may reflect adaptations to local environmental conditions and highlight the functional role of germination and dormancy in community ecology.

2.1 Introduction

Alpine ecosystems harbour plant communities that grow above the natural tree-line and are the only bioclimatic zone found in all latitudes of Earth (Nagy and Grabherr, 2009). These high elevation environments present challenges for plant life, including exposure to strong winds, large temperature fluctuations (from freezing to extreme heat events), short growing seasons and usually nutrient-poor soils. Despite being subjected to extreme conditions, alpine zones contain 4% of higher vascular plant species with a high level of endemism (Körner, 2003).

In the Europe's higher massifs, such as the Alps, snow-protected grasslands dominated by sedge heath and dwarf shrubs are typical (Ozenda and Borel, 2003), though there is a clear landscape contrast between calcareous and siliceous bedrocks. In each bedrock, different environmental filters, for instance the soil physical and chemical parameters (i.e. Ca^{2+} or Al), have strong effects on species composition. Within each bedrock type there is a mosaic of microhabitats occupied by different plant communities, from shelter scan vegetation to tussock grasses (Körner, 2003). In this context, understanding the key aspects of plant regeneration from seed is crucial in order to assess plant mechanisms to the alpine habitats (Fernández-Pascual et al., 2013).

Germination is an irreversible process, and must be timed to occur when the environment is favourable for subsequent seedling establishment (Thompson and Fenner, 2005; Poschlod et al., 2013). Germination timing is controlled by environmental cues (Lambers et al., 2008), chiefly an obligate requirement for soil moisture and variable temperature inputs (Probert, 2000). Dormancy, in addition to germination cues will determine the timing of germination and seedling emergence, and therefore the likelihood of seedling survival. Indeed, germination of some species is inhibited until seeds have received appropriate cues such as light or those related to fire (Baskin and Baskin, 2014). Responses to these cues have evolved into specific germination strategies and dormancy states that reflect species adaptations to different habitats (Willis et al., 2014). Additionally, the conditions experienced by the mother plant during flowering and at dispersal can also influence germination timing of the following generation (Burghardt et al. 2015). For instance, maternal soil nutrient levels are known to affect offspring phenotypic expression so that mother plants grown under high nitrogen concentrations produce seed offspring with weaker levels of dormancy (Wulff et al., 1999).

There is strong environmental control of the plant regeneration life-story stage (e.g dispersal vectors, seed longevity, germination), and examples of environmental features driving

variation in germination strategies include habitat disturbance (Angevine and Chabot, 1979), altitude (Fernández-Pascual et al., 2016), soil nutrients (Hilhorst and Karssen, 2000), density of plant cover (Jankowska-Blaszczuk and Daws, 2007) and chorology (Orsenigo et al., 2015). Seed traits (Pierce et al., 2014) and particularly germination traits (i.e. timing and degree of germination) may play an important role in promoting species coexistence within communities (Kos and Poschlod, 2008; Jimenez-Alfaro et al., 2016). For example, seed weight had been found to be related with seed longevity in the soil seed bank (Cornelissen et al. 2003) and with the species' competitive ability (Tilman, 1994). However, regeneration-related traits remain underrepresented in studies investigating the drivers of vegetation patterns at local and global scales (Kleyer et al., 2008).

In the alpine environment, the large species and microhabitats diversity has resulted in a variety of germination responses and dormancy types, which makes it difficult to define a common "alpine" germination strategy (Körner, 2003; Schwienbacher et al., 2011; Hoyle et al., 2015). For example, although many alpine plants have deep physiological dormancy (Schwienbacher et al., 2011; Sommerville et al., 2013; Baskin and Baskin, 2014) and require light (Jaganathan et al. 2015) and high temperatures for germination (Jumpponen *et al.*, 1999), non dormant seeds (Sommerville et al., 2013) very low temperature requirements and dark conditions (Schwienbacher, 2011) for germination have also been observed.

The steep environmental gradients (e.g. temperature and water) found within a few meters in the alpine habitat (Graham et al., 2012) provide an ideal context to assess changes in germination strategies related with the local environment. Indeed, differences in germination traits have been attributed to slope orientation (Xu et al. 2017), biogeographic provenance (Giménez-Benavides et al. 2005) and species' successional niche (Schwienbacher et al. 2012). The high variability in germination responses identified in alpine plant species has been interpreted as a survival strategy in the face of unpredictable environmental conditions (Kigel, 1995). Therefore, an investigation at habitat level may help to understand the drivers of germination patterns in alpine species and add further insights into their functional significance in community ecology. To this end, here we conducted germination experiments with 53 species inhabiting in the two most representative alpine habitats in Europe, namely those on siliceous (26 species) and calcareous (27 species) bedrocks (European Commission, 2007), using a combination of different pre-treatments (i.e. cold stratification and GA₃) and incubation temperatures (i.e. 25/15°C and 15/5°C). We hypothesized that each habitat would be dominated by different germination behaviours related with local environmental variables, for instance

species' inhabiting in calcareous habitats will show lower germinability when dispersal than those from siliceous habitat because risk of drought is high in the former (Gigon, 1942). To test this, we assessed if (1) in siliceous and calcareous habitats species show common germination strategies; and (2) germination traits can be influenced by the species' main microhabitat (grasslands, rocky, heaths and species occurring in more microhabitats) and by their chorology (Arctic-Alpine and Continental) (*sensu* Passalacqua, 2015).

2.2 Material and methods

2.2.1 Study system

The studied species belong to the Natura 2000 habitat types '6230 - *Nardus*-rich species grasslands'; and '6170 - Alpine and subalpine calcareous grasslands' (92/43/CEE "Habitat" Directive classification) (European Commission, 2007). Habitat #6230 occurs in nutrient-poor soils with acidic pH, which increase the availability of ions like A^{1+} and H^{+} . This habitat is formed on various types of siliceous rocks (mainly crystalline slides and granite, and volcanic rock). Its frequent plant species include *Nardus stricta*, *Carex curvula* and *Arnica montana* (Gennai et al., 2014). Meanwhile, habitat #6170 is characterized by nutrient-rich soils and alkaline pH, which increase the availability of ions like Ca^{2+} . It is formed on calcareous bedrocks and its typical plant species include *Sesleria caerulea*, *Dryas octopetala* and *Phyteuma orbiculare* (European Commission, 2007). Calcareous habitats are drier than siliceous because of their lower water holding capacity (Körner, 2003). Moreover, calcareous soils hold a higher biodiversity than siliceous in which many endemisms, rarities and species with high biogeographical value occur (Pawłowsky, 1970). For simplicity, each habitat is referred to hereafter by its soil type (siliceous and calcareous) or code (#6230; #6170).

2.2.2 Species selection

Fifty-three species, representing 19 plant families, were chosen based on their occurrence and abundance in these two habitats. From the 53 species included in this research, 26 species were collected in the siliceous habitats and 27 were from the calcareous bedrocks. Inside each habitat, different microhabitats such as rocky, grasslands or heaths places were identified; for this reason, species were collected also having in account their main microhabitat. Species' chorology and microhabitat derived from Aeschimann et al. (2004) (Supplementary Material 2.1). Chorology (*sensu* Passalacqua, 2015) was considered as the area of distribution of the species, while microhabitat was defined as the most common place for species occurrence:

grasslands, rocky, heaths and generalist (i.e. species occurring in more than three microhabitats).

Freshly matured seeds were collected from about 50 to 100 plants of each of the 53 species in summer and autumn 2015 (i.e. August to October) in the following Sites of Community Interest (SCI): Val Viola Dosde (46° 24'N, 10°12'E) and Passo dello Stelvio (46° 32'N, 10° 25'E), both located in the Alps of Lombardy (Sondrio, northern Italy). From each plant, approximately 20-100 seeds were collected depending on the species. After collection, seeds were cleaned, pooled and stored at room temperature until the beginning of the experiments, which occurred within 2 weeks after the collection. This methodology was chosen because we were interested in defining seeds' primary dormancy and to avoid any possible change in the germination and/or dormancy response induced by holding seeds in the lab (Baskin and Baskin, 2014). Indeed, after ripening can have different effects on species' germination, increasing the final germination percentage for *Avena fatua* (Johnson and Dyer, 2000) or decreasing it for *Eucalyptus pauciflora* (Beardsell and Mullett, 1984).

2.2.3 Germination experiments

Laboratory experiments involved subjecting sown seeds to three cold stratification periods of 0, 3 and 5 months (hereafter referred to as 0, 3 and 5 CS) at 0 °C in complete darkness. All germination tests started the same day. After each interval, seeds were incubated for germination at two alternating temperatures to simulate summer (25/15°C) and autumn/spring (15/5°C) daily field conditions, reflecting conditions during the most suitable period for seedling emergence at the species growing sites (Mondoni et al., 2012). For each species and treatment, three samples of 20 seeds each were sown on 1 % distilled water-agar in 50 mm diameter Petri dishes. Following the Baskin and Baskin seed dormancy classification (2014), one of the important distinguishing features of the degree of physiological dormancy is whether seeds respond to gibberellins (GA₃). Consequently, seeds were also incubated at 25/15°C with 250 mg l⁻¹ of GA₃ incorporated into 1% agar. Plates were checked for germination monthly during the cold stratification and weekly (for five weeks) during the germination at 25/15 and 15/5°C. Seeds were scored as germinated when the radicle protruded >2mm. At the end of the experiments, non-germinated seeds were cut-tested to confirm their viability. Empty seeds or fungus infected with fleshy or dark embryo were considered non-viable. Then, the final germination percentage (FGP) and the time to 50% of germination (T₅₀) were calculated excluding non-viable seeds. All germination tests were carried out in temperature and light controlled incubators (LMS 250A, LMS Ltd, Sevenoaks, UK) using a 12-h daily photoperiod

(photosynthetically active radiation $40 - 50 \mu\text{mol m}^{-2} \text{s}^{-1}$). The results of the experiments were used to create a germination matrix in which each species was assigned the 13 or 15 germination outputs (see Supplementary material 2.2), representing all the species' mean (or GA₃ aside) FGP and T₅₀ of each treatment. T₅₀ of each treatment was calculated using R version 3.3.2. The log-logistic, a dose-response model, is fitted to the cumulative germination data to calculate the time needed to reach 50% of germination.

2.2.4 Habitat-related seed germination traits

The treatments (i.e. including 0 CS, 3 CS, 5 CS and GA₃ at 25/15°C and 15/5°C) were used to assess differences in the FGP between habitats (i.e. siliceous and calcareous). In these models, mean FGP of each specie and treatment with a logit transformation were fitted with different Phylogenetic Least Squared Regression (PGLS) (Grafen, 1989) implemented in the package “nlme” (Pinheiro et al., 2015) against habitat. PGLS was used because it incorporates an expected model of evolution and phylogeny into the variance-covariance matrix (Kraft et al., 2015). This accounts for the non-independence among observations (i.e., species) due to closely related species having similar traits values (Harvey and Pagel, 1991). The phylogenetic signal (λ) and the regression parameters were calculated simultaneously by a maximum-likelihood (ML) estimation (Revell, 2010). The value of λ ranges from 1 to 0. High values of λ indicate phylogenetic dependence among observations as predicted by a Brownian evolution model, whereas values close to 0 indicate phylogenetic independence among observations

Moreover, germination strategies were assessed within habitats using cluster-analysis computed with the Euclidean distance matrix using the 15 germination outputs (i.e., T₅₀ and FGP) and k-means algorithm from the Factoextra package in R (Kassambara, 2015). The appropriate number of cluster was chosen considering the results of different combinations of number of clusters, distances measure and clustering methods in both habitats with the package NbClust (Charrad, 2015). Finally, 4 germination strategies within each habitat were compared and plotted using two Principal Component Analysis (PCA) with the “FactoMineR” package (Le et al., 2008).

2.2.5 Seed dormancy classes

To assign each species under a seed dormancy class (*sensu* Baskin and Baskin 2014), information related to seed coat permeability and embryo type were obtained from the literature (Martin, 1946; Baskin and Baskin, 2007). To determine physiological and morphophysiological dormancy level, Generalized Lineal Mixed Models (GLMM) with

binomial error structure and logit link function were built for each species. In these models, seed germination proportion (i.e. number of germinated seeds out of number of viable seeds of each species) was the response variable, whereas, dormancy-breaking treatment [4-level categorical variable including 0 CS, 3 CS, 5 CS and GA₃], temperature (2 level categorical variable, including 15/5, 25/15 °C) and their interaction were the explanatory variables. Finally, replicates were treated as a covariable. According to the data collected from the literature and the germination response observed here, species were assigned a type and class of dormancy following the Baskin and Baskin classification (2014) and Silveira's diagram (2013). For example, when the FGP of seeds incubated at 3 or 5 CS were significantly higher than FGP at 0CS, the embryo was fully developed at dispersal and the seedcoat was permeable, seeds were considered PD (for further information about the classification criteria used see Supplementary material 2.3 a and b).

2.2.6 Phylogenetic comparative analysis

We conducted a comparative phylogenetic analysis to evaluate the influence of microhabitat and chorology on species germination behaviour. First, the germination matrix, in which 13 germination outputs were included (i.e. FGP and T₅₀ for all treatments with GA₃ aside), was reduced using a new Principal Component Analysis (PCA). Then, species scores in the PCA Axis I and II were regressed against the species chorology and microhabitat using PGLS. Finally, the best model to explain each axis was selected using Akaike's Information Criterion (AIC) (Burnham et al., 2011).

2.2.7 Seed weight.

The weight of 50 seeds (g) collected during the growing season 2016 was measured. Mean seed weight for each species was obtained from 5 replicate weights. Weight was log-transformed to proceed with statistical analysis. Firstly, correlations between seed weight and Axis I, Axis II, FGP 0M 15/5°C and FGP 0M 25/15°C were assessed fitting lineal models. Secondly, differences in species' seed weight between habitats and microhabitats were compared using PGLS.

2.3 Results

2.3.1 Habitat-related seed germination traits

Seed germination varied across habitats for some treatments (Table 2.1). The PGLS revealed that species from the siliceous habitat had a significantly higher FGP than those from calcareous habitat in the 0CS and 15/5°C treatment, though no differences in FGP were found between habitats in the other treatments (i.e., 0CS and 25/15°C, 3CS and 5CS and 15/5°C, 3CS and 5CS 25/15°C, GA₃). Further analysis showed that FGP during cold stratification was higher in species from the calcareous habitat (i.e. 12%) compared to those from the siliceous (i.e. 6%), though in each habitat 14 and 12 species (i.e. calcareous and siliceous habitat respectively) germinated during the cold stratification (i.e. 0°C and darkness). (see Supplementary material 2.4).

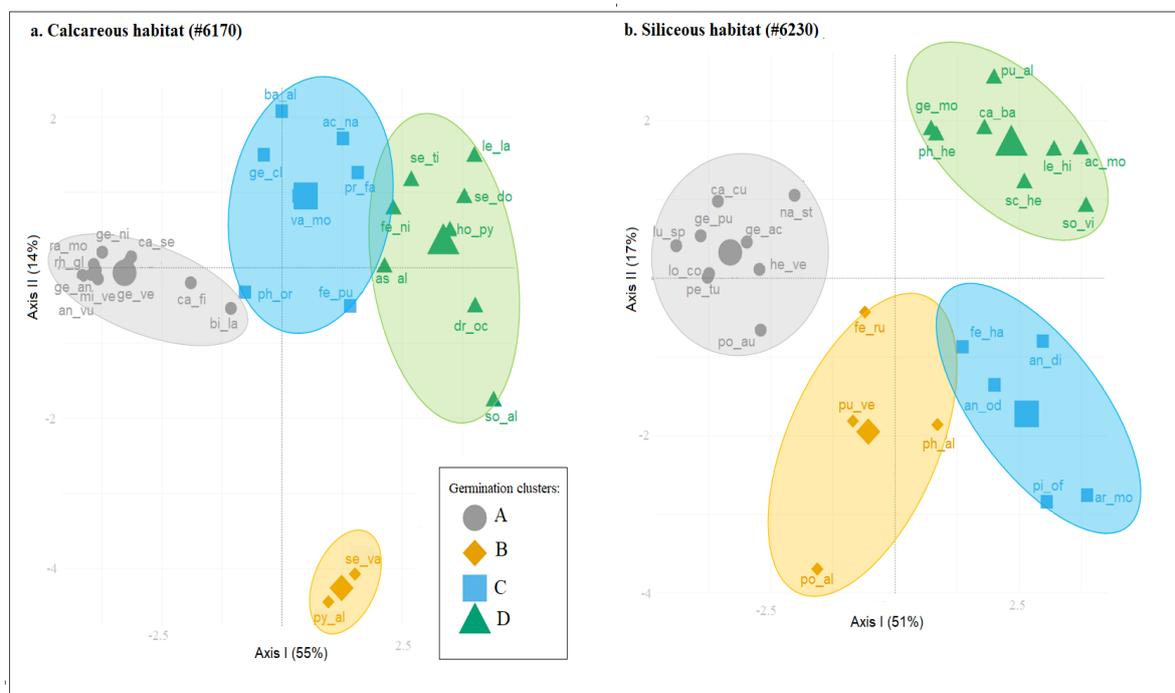
Table 2.1. Results from the Phylogenetic least squares relation between habitat and FGP. Significant ($P < 0.05$) values in bold character

Response variable	Λ	FGP calcareous grassland (%)	in FGP in siliceous grassland (%)	p.value
FGP during cold stratification	1.00	12	6	<0.001
FGP - 0CS 25/15	0.67	43	46	0.67
FGP- 0CS 15/5	1.02	14	23	<0.001
FGP-3CS 25/15	0.56	51	60	0.65
FGP-3CS 15/5	0.43	57	57	0.95
FGP-5CS 25/15	0.70	87	88	0.14
FGP-5CS 15/5	0.37	58	59	0.63
FGP-GA ₃	0.28	57	93	0.53

The habitat-PCA results were similar. Axis I explained 54.7% and 51.3% of the total variance for calcareous and siliceous habitat respectively. Axis I was correlated with all treatments, although the relationship was positive with the FGP and negative with T_{50} , summarizing the capacity to germinate for both habitats. Axis II explained 14.2% and 17.2% of the variance for calcareous and siliceous habitats respectively. It was mainly positive correlated with FGP 0CS in the calcareous habitat and with FGP 0CS and FGP during cold stratification in the siliceous. Axis II explained the capacity of the species to germinate right after dispersal.

Moreover, in the calcareous habitat, the cluster analysis (Figure 2.1a) revealed four major clusters (i.e., A, B, C and D; Table 2.2). ‘Cluster A’ represents the lower germinators (i.e. 10 species). ‘Cluster B’ portrays species whose germination decreased after cold stratification, while it was high and rapid on fresh seeds (i.e., 2 species). ‘Cluster C’ (i.e., 7 species) includes the species with slow germination, occurring mostly after the cold stratification. Finally, ‘Cluster D’ (i.e., 8 species) represents species with high and rapid germination capabilities, mostly showing only low FGP immediately after dispersal at the low incubation temperature (15/5°C) and/or during the cold stratification (Table 2.2).

Figure 2.1. Principal component analysis (PCA) representing the main two axis of variation on the germination patterns. Each spot represents a species indicated by the two first letters in the genus and name. The collections are clustered into four main groups according to their germination strategy. Confidence ellipses represent 0.80 intervals of confidence around the species per cluster a) Calcareous bedrock. b) Siliceous bedrock.



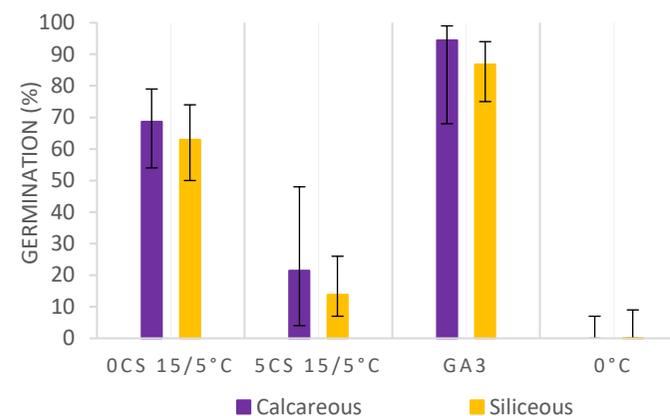
Overall, the cluster analysis (Figure 2.1b) displayed a different position of the germination groups and a higher within-group variability in the siliceous habitat compared to that of the calcareous habitat. Hence, in siliceous clusters species germination responses is more diverse than in the calcareous clusters. Particularly, main differences were observed in ‘Cluster A’ describing again the lower germinators, but also some others that germinated only with GA₃ (i.e., 9 species) and in ‘Cluster C’ (i.e., 5 species) describing species with high and rapid

Table 2.2 Germination clusters (A, B, C and D) and species belonging to each them divided by habitat provenance. The germination response was summarized using barplots representing FGP (Final germination percentage) scored in some of the conditions tested of one representative specie (in bold) from each germination bedrock, species inhabiting in calcareous habitats in purple and siliceous species' in yellow.

Clusters	Species		Germination response																					
	Siliceous	Calcareous																						
A	<i>Carex curvula</i> , <i>Gentiana acaulis</i> , <i>Gentiana punctata</i> , <i>Helictochloa</i> <i>versicolor</i> , <i>Lotus corniculatus</i> , <i>Luzula spicata</i> , <i>Nardus stricta</i> , <i>Pedicularis tuberosa</i> , <i>Potentilla</i> <i>aurea</i>	<i>Anthyllis vulneraria</i> , <i>Biscutella</i> <i>laevigata</i> , <i>Carex firma</i> , <i>Carex</i> <i>sempervirens</i> , <i>Gentiana nivalis</i> , <i>Gentiana verna</i> , <i>Gentianella</i> <i>anisodonta</i> , <i>Minuartia verna</i> , <i>Rhinanthus glacialis</i> , <i>Ranunculus</i> <i>montanus</i>	<table border="1"> <caption>Germination response data for Cluster A</caption> <thead> <tr> <th>Condition</th> <th>Calcareous (%)</th> <th>Siliceous (%)</th> </tr> </thead> <tbody> <tr> <td>0CS</td> <td>~8</td> <td>~8</td> </tr> <tr> <td>15/5°C</td> <td>~15</td> <td>~12</td> </tr> <tr> <td>5CS</td> <td>~18</td> <td>~15</td> </tr> <tr> <td>15/5°C</td> <td>~22</td> <td>~18</td> </tr> <tr> <td>GA3</td> <td>~12</td> <td>~48</td> </tr> <tr> <td>0°C</td> <td>~5</td> <td>~5</td> </tr> </tbody> </table>	Condition	Calcareous (%)	Siliceous (%)	0CS	~8	~8	15/5°C	~15	~12	5CS	~18	~15	15/5°C	~22	~18	GA3	~12	~48	0°C	~5	~5
Condition	Calcareous (%)	Siliceous (%)																						
0CS	~8	~8																						
15/5°C	~15	~12																						
5CS	~18	~15																						
15/5°C	~22	~18																						
GA3	~12	~48																						
0°C	~5	~5																						

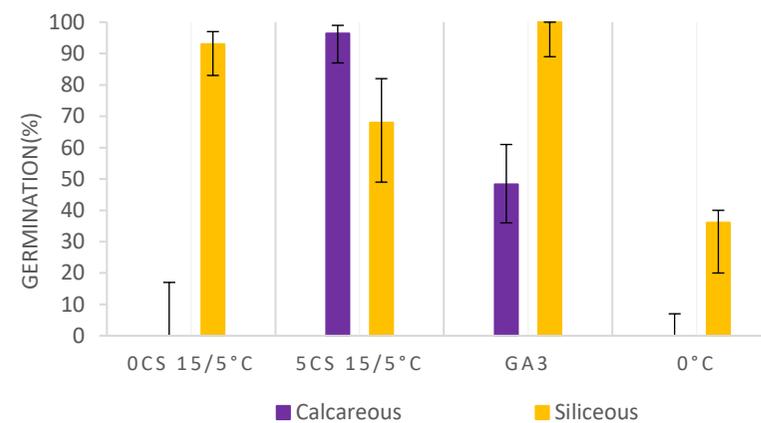
B *Festuca nigrescens*, *Phleum raethicum*, ***Poa alpina***, *Pulsatilla vernalis*

Polygala alpina, ***Sesleria caerulea***

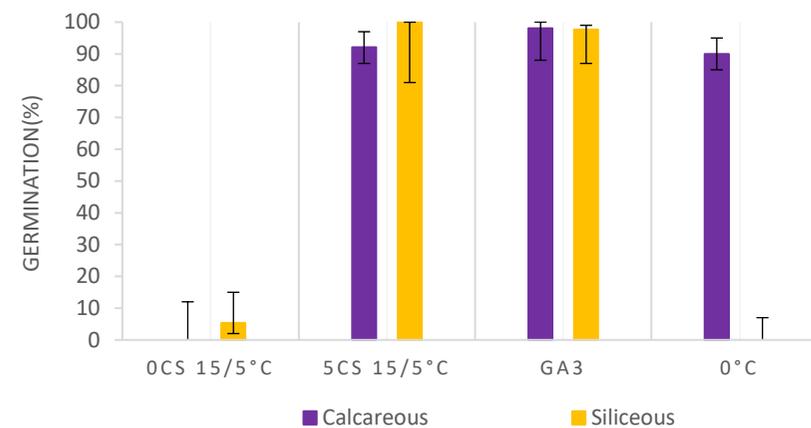


C *Antennaria dioica*, *Anthoxanthum odoratum*, ***Arnica montana***, *Festuca halleri*, *Pilosella officinarum*

Achillea nana, *Bartsia alpina*, *Festuca pumila*, ***Gentiana clusii***, *Phyteuma orbiculare*, *Primula farinosa*, *Valeriana montana*



D	<i>Achillea moschata</i> , <i>Campanula barbata</i> , <i>Geum montanum</i> , <i>Leontodon hispidus</i> , <i>Pulsatilla alpina</i> , <i>Scorzoneroides helvetica</i> , <i>Phyteuma hemisphaericum</i> , <i>Solidago virgaurea</i> .	<i>Aster alpinus</i> , <i>Dryas octopetala</i> , <i>Festuca nigricans</i> , <i>Horminum pyrenaicum</i> , <i>Leontopodium alpinum</i> , <i>Senecio doronicum</i> , <i>Serratula tinctoria</i> , <i>Soldanella alpina</i>
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0CS 15/5°C. FGP scored of seeds sown after 0 months of cold stratification (or fresh seeds) at 15/5°C incubation temperature.

5CS 15/5 °C. FGP scored of seeds sown after 5 months of cold stratification at 15/5 °C incubation temperature.

GA3°C. FGP scored of seeds seeds sown with 250 mg/L of gibberlic acid at 25/15°C incubation temperatura

0°C. FGP scored of seeds sown at 0°C and dark conditions during 5 months.

germination under all conditions (not present in the calcareous habitat), including the emergence during cold stratification. Finally, the species' categories in Clusters B (i.e., 5 species) and D (i.e., 9 species) were similar between the habitats (Table 2.2).

2.3.2 Dormancy class and level

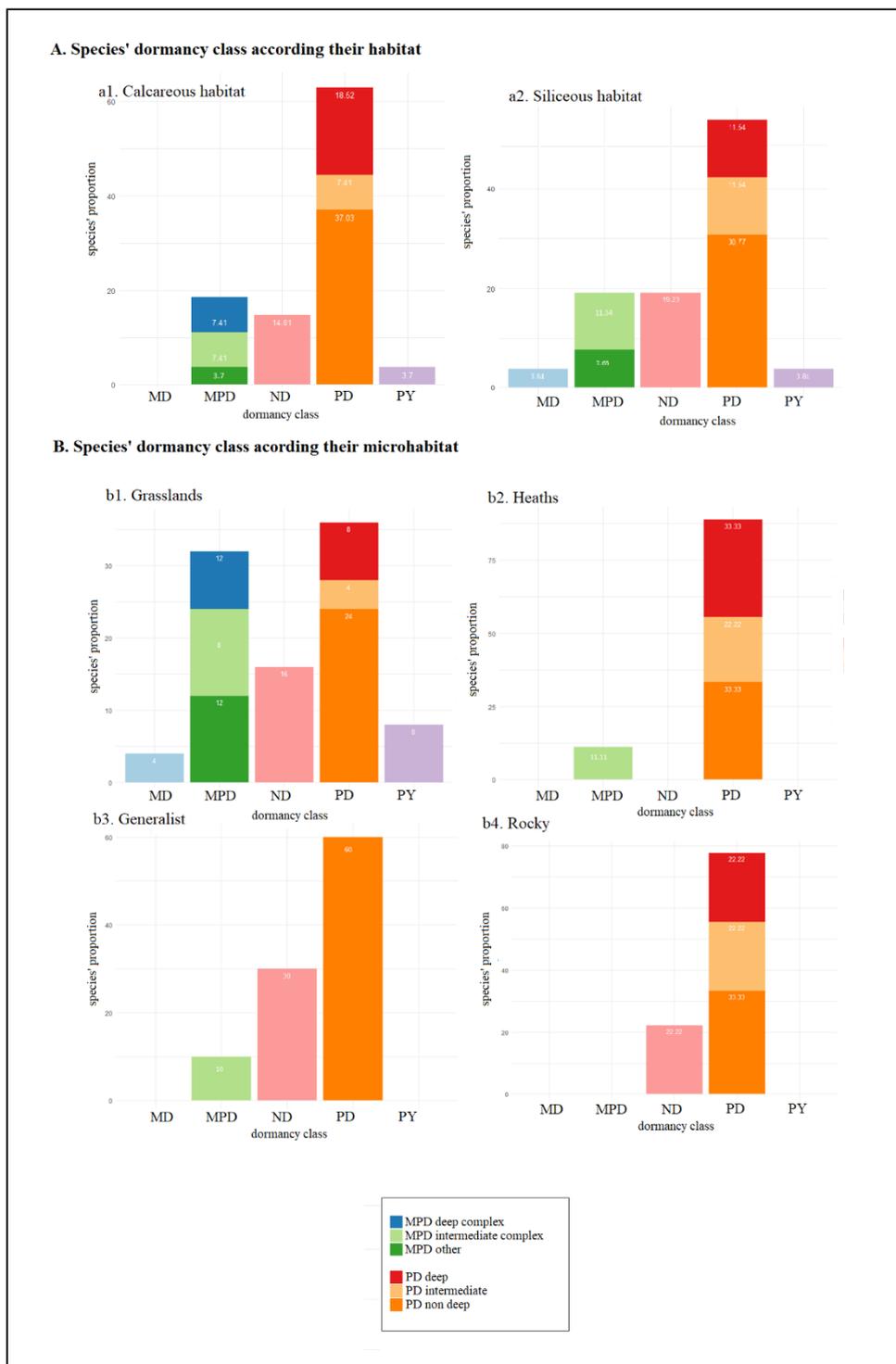
Overall, most of the species produced dormant seeds at dispersal (See Supplementary material 2.3a and b, as only nine species (<20% of those tested) were found to have predominantly non-dormant seeds. The most representative dormancy class was PD (32 out of 53), and out of those, the non-deep level was the most abundant (19 out of 32), followed by deep (7 out of 32) and the remaining six species were classified in the intermediate level. Out of eleven species that had undifferentiated or underdeveloped embryos at dispersal, only one (*Pulsatilla vernalis*) was considered to have MD, while the others showed MPD; five species (*Pulsatilla alpina*, *Gentiana clusii*, *Gentiana acaulis*, *Gentiana verna* and *Campanula barbata*) were classified under the intermediate complex MPD and two species under deep complex type (*Gentiana nivalis* and *Bartsia alpina*). Further studies are needed to classify the remaining three species (i.e. *Gentianella anisodonta*, *Pedicularis tuberosa* and *Gentiana punctata*) to a MPD level. Finally, only two species (*Lotus corniculatus* and *Anthyllis vulneraria*) were found to be physically dormant with an impermeable seed coat (See supplementary material 2.3b).

Dormancy was compared between both habitats and microhabitats (Figure 2.2). At habitat level, no differences were detected in the proportion of species with dormancy class and level. However, differences were found between microhabitat, with species occurring on heaths producing only dormant seeds (c. 89 % PD and 11% MPD), followed by grasslands showing 84% of species with dormant seeds (36% PD; 32% MPD, 8% PY and 4% MD), rocky with c. 77 % of species showing PD and the generalist (60% PD and 10% MPD). Additionally, generalist species showed only the lowest intensity of dormancy (i.e. non-deep PD), while in the other microhabitats there was always a given degree of more intense dormancy state, either deep/intermediate-PD or deep/intermediate/complex-MPD.

2.3.3 Phylogenetic comparative analysis

Germination traits were summarized using a PCA (Figure 2.3), in which the two-principal axes explained 73.65% of the variance (See Supplementary Material 2.5). Axis I (representing 57.6% of the variance) corresponded to the ability to germinate and is referred to here as “germinability”. It was positively correlated with all the FGPs and negatively with the T₅₀,

Figure 2.2 Proportion of dormancy class and level of the target species (MD (Morphological dormancy), MPD (Morphophysiological dormancy), PY (Physical dormancy), PD (Physiological dormancy) and ND (Not dormant) within a) each habitat (from right to left, calcareous and siliceous bedrocks) b) each microhabitat (from right to left and from top to down: heaths, rocky, generalist, grassland).



and it was mostly explained by the 3 and 5CS, treatments across both germination test temperatures (See Supplementary Material 2.5). Hence, the species with slow and low germinations were separated from the species with fast and quick germinations; for example, *Carex curvula* occupies the left part of Axis I, while *Arnica montana* is on the right. Axis II, explaining 16% of the variance, was negatively correlated with the FGP of OCS and positively with T_{50} of OCS (See Supplementary Material 2.5). Hence, species which had the ability to germinate immediately following dispersal, such as *Pilosella officinarum*, appear on the lower part of the plot, whereas species which did not germinate immediately following dispersal, such as *Gentiana clusii*, appear in the top part of the plot. Therefore, Axis II is considered here as “germinability after dispersal”.

The PGLS were fitted using both Axis I and II and the best model was selected using the AIC criteria. For this reason, when the AIC decreased, no significant differences between models were detected and the levels' intercept were similar, the levels were grouped together, following Crawley (2013). So, Chorology resulted in a two level factor [Arctic-alpine (including Artic-alpine, Alpine and South-Europe mountain origin chorotypes) and Continental (including Eurosiberian, Euroasiatic and Europe chorotypes)] and Microhabitats for Axis I in a three level factor [Grasslands-Heath (including species inhabiting grasslands and heaths), Rocky and Generalist].

The final models demonstrate that species Axis I scores significantly differ based on the species' microhabitat occurrence (Table 2.3); and the Axis II scores as a function of the chorology and microhabitats (Table 2.3). The phylogenetic signal (Pagel's λ) associated with the regression residuals was close to 0.6 for Axis I (or germinability) (Table 2.3), indicating moderate phylogenetic dependence in the relationship. λ was 0.34 for Axis II (Table 2.4), showing more phylogenetic independence among observations than the previous one. Species with high Axis I scores (See supplementary material 2.1) belong mostly to the generalist (Figure 2.3a). In contrast, species with low Axis I scores occur mostly in grasslands. The species with high Axis II scores (See supplementary material 2.1) mostly occurred on heath and have an Artic-Alpine chorotype (Figure 2.3b). Meanwhile the species with low Axis II scores are mostly generalists and have a continental chorotype.

2.3.4 Seed weight

Overall, species' weight differed greatly among species (Supplementary material 2.2). The magnitude of seed weight ranged from 0.00132 g (i.e. *Gentiana nivalis*) to 0.29g (i.e. *Lotus corniculatus*), with a mean of 0.0578 g per 50 seeds.

Table 2.3. Phylogenetic least squared correlations between germination traits and ecological traits. Significant ($p>0.05$) values in bold character and differences among levels of a factor indicate by different letters.

Models	Factor	Level	Mean	SE	p.value
Germinability ~ microhabitat $\lambda = 0.66$ AIC=242.85	Microhabitat	Generalist	1.196a	1.09	
		Grassland-Heaths	-0.850b	0.87	
		Rocky	0.403ab	1.11	0.05
Dormancy ~ microhabitat + Chorology $\lambda = -0.34$ AIC: 181.22	Microhabitat	Generalist	-0.903a	0.52	
		Heath	0.964b	0.48	
		Grasslands	-0.377a	0.37	
		Rocky	0.039ab	0.48	0.001
	Chorology	Continental	-0.878a	0.47	
		Artic-Alpine	0.074b	0.35	0.039

The individual lineal models assessing lineal relationships between seed weight and the two-principal axis (i.e. Axis I and Axis II) and the log-transformed FGP resulted on weak relationships (see Supplementary material 2.6). No relations between mean seed weight and habitat or microhabitats were detected (see Supplementary material 2.6).

2.4 Discussion

2.4.1 Habitat-related seed germination traits

Our results show that habitat provenance has a significant effect on FGP and on the germination strategies (i.e. clusters) in each bedrock, indicating the presence of habitat-related regenerative strategies. In particular, species from 'Cluster C' in the calcareous grasslands showed a slow germination and mostly after cold stratification (i.e. see middle-upper part of Figure 2.1a). Consequently, these species would germinate mostly in spring when there is a lower risk of heat stress, with the remaining ungerminated seeds forming a persistent soil seed bank. Topsoil desiccation and temporary stresses such as wind erosion and hyperthermia in summer (Gigon, 1942) are common in calcareous habitats (Kammer and

Mohl, 2016) and may strongly affect seedling establishment (Marcante et al., 2014). This may explain why species from the calcareous grasslands displayed a significantly higher FGP during the cold stratification period, when water availability is high. As a result, temporally spread winter emergence would allow some seedlings to reach the summer drought period at a developed stage; for example, young plants would have a deeper root system and deeper soil layers rarely dry up (Körner, 2003). Conversely, in siliceous grasslands, characterised by lower risk of drought, the germination strategy found in ‘Cluster C’ showed no restrictions, germinating to high levels in all conditions tested (i.e. see lower-right part of Figure 2.1b). These results indicate that species from siliceous soils are able to stagger their germination throughout the whole year depending on the environmental conditions. For instance, if summer temperatures are warm enough, a proportion of the population would be able to germinate. However, in years with cooler summers, germination can be postponed until after snowmelt, with a proportion of seeds germinating under the snow. This germination plasticity may potentially increase the regeneration capacity of a species, albeit as a result of depletion of the soil seed bank. Moreover, soil water availability during seed development and maturation is also known to have large influence on seed germination requirements, via maternal effects (Lu et al., 2016). Therefore, the possibility that habitat dormancy differences may be related with the environmental heterogeneity of the growing site cannot be ruled out. Interestingly, ‘Clusters A, B and D’ show common germination strategies in both bedrocks, having similar position in the PCA axis (Figure 2.1). The low germination of the species belonging to ‘Cluster A’ may indicate that cold stratification is not always effective in promoting germination for alpine species or that appropriate cues for germination were not met (Baskin and Baskin, 2006; Donohue, 2010). The low germination of alpine species under laboratory conditions has been interpreted as a mechanism to ensure the formation of a persistent soil seed bank (Shimono and Kudo, 2005; Mondoni et al., 2012), as an ecological strategy to the low chance of establishment in these environments (Schwienbacher et al., 2010; Erschbamer et al., 2008), due to summer drought/heat and early autumn/spring frost episodes (Graae et al., 2009; Marcante et al., 2012, 2014).

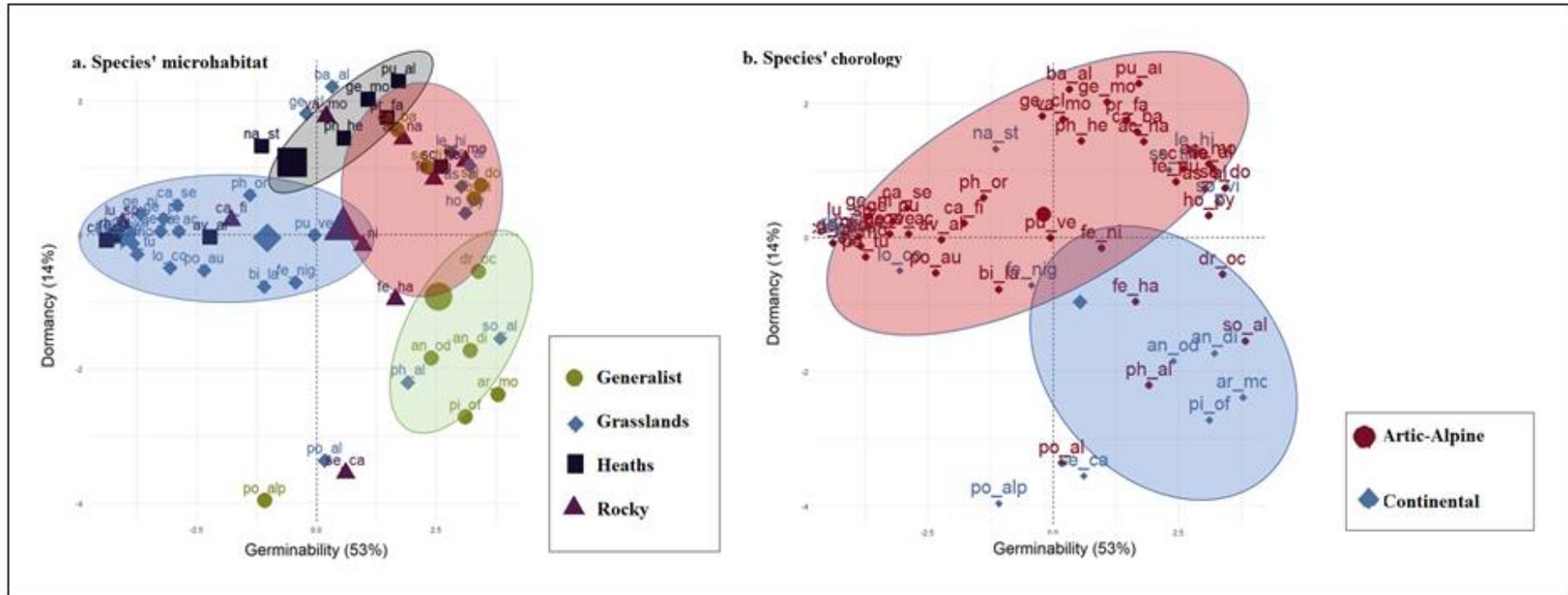
The strategy summarized for ‘Cluster B’ is represented by species with non-dormant seeds, which germinate after dispersal, in late summer or autumn. These species probably remain in the soil surface if they required light for germination or they are species able to germinate under dark conditions. In addition, for some species (i.e., *Sesleria varia*, *Polygala alpina*, *Poa alpina*, *Pulsatilla vernalis* and *Festuca rubra*) belonging to this germination strategy, cold

stratification significantly reduces FGP compared with 0 CS, i.e., potentially induces deeper dormancy. Despite the fact that autumn germination in alpine habitats has often been considered disadvantageous, recent studies have shown that a high number of autumn-emerged seedlings could survive winter on glacier forelands (Marcante et al., 2012; Mondoni et al., 2015). Autumn germination may be advantageous for species that produce seeds with short longevity and unlikely to form a persistent seed banks, and represents an ecological advantage that presumably ensures seedlings are well placed to grow quickly when temperatures begin to rise in early spring. Finally, species belonging to ‘Cluster D’ postponed germination until late winter or early spring. Overall, seed germination and seedling establishment of alpine plants tends to occur rapidly after snowmelt (Körner, 2003; Schwienbacher et al., 2011), when there is lower risk of frost and temperatures rise fast (Rosbakh and Poschlod, 2015). These conditions are favourable for seedling recruitment as the plants have the entire growing-season to reach their optimal size for overwintering (Billings and Mooney, 1968) and early germination has a strong competitive advantage (Grime, 2002).

2.4.2 *Phylogenetic comparative analysis*

Germination traits (Axis I and II) were weakly correlated with species’ weight and no differences on mean seed weight detected between habitats and microhabitats (see Supplementary material 2.6). Therefore, seed weight did not contribute on explaining local vegetation patterns here, though, it is the main regenerative traits used in community ecology publications (Jiménez-Alfaro et al. 2016). On the other hand, germination traits were strongly correlated with the microhabitat of the species occurrence and with species’ chorotype (Figure 2.3), highlighting that such factors may importantly contribute to affect plant regeneration strategies. In particular, species inhabiting in heaths showed high Axis II scores, indicating that germinability is high only after dormancy is broken (i.e. after cold stratification), hence that most of these species have dormant seeds. Consistently, all species from heaths were classified as having dormant seeds, with about one third showing deep PD. Moreover, scores levels of Axis II were the lowest in the generalists, as well as the presence and the level of dormancy (60% non-deep PD; 10% MPD; Supplementary material 2.2b). Our results also showed differences among Axis I scores, with generalist species having higher scores than those from grasslands, confirming that dormancy state is weak in the former. Accordingly, generalist

Figure 2.3. Principal Component Analysis (PCA) representing the main two axes of variation on the germination patterns ('germinability' and 'dormancy'). Each spot represents a species indicated by the two first letters in the genus name and the specific epithet. The species are coloured according to: a) Species' microhabitat occurrence, b) Species' chorology.



species showed the highest percentage of ND seeds (30%) and grasslands were among the most dormant, showing 84% of species with dormant seeds, including deep PD and MPD. Following the view of Grubb (1977) and subsequent observations (Huang et al. 2016), which highlighted the ecological role of the regeneration niche (including germination traits) in driving plant distribution, our results show that dormancy presence and levels were strongly related to the species occurrence in the microhabitats. This is an interesting and novel observation, which help explaining the large diversity of germination and dormancy responses in alpine environments and add novel insights to their possible functional role as drivers of species distribution in alpine environment (*sensu* Jimenez-Alfaro et al., 2016).

Since dormancy is a mechanism of plants to synchronize germination with a suitable moment for seedling recruitment (Baskin and Baskin, 2014), the different germination behaviours found here indicate that recruitments from seeds follow different patterns in each microhabitat. However, such possibility cannot be fully understood without a detailed investigation of the microclimate in each habitat, population density in each microhabitat and/or *in situ* germination evidences. Therefore, any explanation of the possible environmental cue affecting the major germination strategy in each microhabitat is purely speculative, though worthy of comments. For example, plants inhabiting wind-exposed and steep places such as often happen in sedge heaths (Nagy and Grabherr, 2009) would expose seedlings to high risk of frost and desiccation stress in the case of autumn emergence. Consequently, plants inhabiting in heaths produce seeds capable of maintaining deep dormancy until winter has passed. On the other hand, in snowbeds, where some generalists occur, thermal buffering under constant snow cover protects any emergence seedlings during winter (Schaberg et al., 2008), which may explain why dormancy was weaker in this microhabitat. Again, the high germination capacity of generalist species may increase the likelihood of seedling recruitment in different microhabitats. Indeed, with wider germination conditions may be exposed to more spatial and temporal establishment opportunities and occupied different types of environments (Thompson and Ceriani, 2003). Meanwhile, the narrower and lower germination conditions showed by most of the species occurring in grasslands may indicate that the appropriate germination cuing were lacking or that longer soil seed banks are expected in these species. Seeds of alpine plants have been shown to form persistent soil seed banks (Schwienbacher et al., 2010), providing an ecological advantage by avoiding unfavourable environmental conditions for seedling establishment (Ooi, 2012). It should be clear that while we used the term “species” here to highlight species-specific

germination strategy linked the two habitats, any interpretation in an evolutionary context must be considered limited to the individuals and their populations here studied.

Finally, in our study arctic/alpine chorotypes (arctic-alpine, alpine and south-europe-montane) gave higher dormancy scores (Axis II) than continental species. This difference supports the hypothesis that seed dormancy is partly influenced by the natural life-history of plants (Schaal and Leverich, 1981) and, hence, by the environmental conditions experienced in the past. In this case, selection pressure on montane areas acts to prevent germination after dispersal (e.g., through a higher dormancy state) and decrease the probability of encountering subsequent unfavourable growth conditions (Probert, 2000), which may explain our findings.

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Chapter 3

Seed germination niches contributes to limit some plant species distributions to calcareous or siliceous alpine bedrocks



The work presented in this chapter has been submitted for publication:

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Abstract.

Functional plant traits are used in ecology for explaining population dynamics in time and space. Six vegetative traits have been used to explain most of the plant-global variability. However, because germination niche is an essential moment in alpine plant life cycles and it is under strong environmental control, we hypothesised that germination traits are critical to an assessment of alpine plant distributions. Germination traits of seven closely related species from calcareous and siliceous habitats were characterised in the laboratory including base, optimum and ceiling temperatures (T_b , T_o , T_c), base water-potential (Ψ_b) and the pH range of germination. Species' vegetative traits (specific leaf area, leaf area and leaf dry matter content) were defined by querying the TRY-database. Traits and habitat similarities and dissimilarities were assessed. Thereafter, species were clustered by habitat provenance in the germination and vegetative traits multivariate space as defined using two separate PCA's. Species from calcareous habitats showed significantly higher T_b , lower Ψ_b and lower capacity to germinate under acidic pH than species from siliceous habitat. Conversely, high T_o and T_c , narrow temperature range for germination at dispersal and similar values for vegetative traits were common in both habitats. Germination traits were clustered by species habitat provenance, meanwhile vegetative traits overlapped habitat provenance. The high vegetative traits similarities indicate small differentiations in adult plants between habitats. Conversely, the separate habitat clusters when using germination traits and the significant differences detected when assessing habitat dissimilarities, suggest that species occurrence in the two habitats may be limited by germination constraints.

3.1 Introduction

Studying species occurrence can inform and predict global and local species plant distribution, and community assembly related to climate change (Diaz and Cabido 2001) or ecological restoration (Merritt *et al.* 2011). In this context, studying plant functional traits is a useful approach to summarize and simplify the structure or function of plant communities rather than considering each species individually and the complexity of their interactions (McGill *et al.* 2006). Furthermore, traits can be useful to explain variance in community structure (de Bello *et al.* 2012). Recently, global plant variability was summarized in a plane using only six adult plant-functional traits (Díaz *et al.* 2015) (i.e. adult plant height (H), stem specific density (SSD), leaf size expressed as leaf area (LA), leaf mass per area (LMA), leaf nitrogen area per unit mass (N_{mass}) and diaspore mass(SM)). However, deciding which are the key-plant functional traits is key to develop predictive frameworks relating to important topics such as community structure (Funk *et al.* 2016) , response to climate warming (Ortu *et al.* 2012; Valladares *et al.* 2014), invasive species (Gross *et al.* 2015) or to biotic or abiotic disturbance.

The convergence of functional traits in species occupying similar environments can be considered an adaptation to environmental filtering (Keddy 1992). For example, alpine species are characterized by low seed mass (Venn *et al.* 2011) and leaf nitrogen content per unit of mass (N_{area}) and high leaf mass - area ratio (LMA) (Read *et al.* 2014). These traits have been associated with high stress tolerance and a low competitive ability (Wright *et al.* 2004), providing some adaptations to harsh abiotic stress. Alpine environments are characterized by short growing seasons, freezing events and extreme temperatures; environmental conditions that constrain alpine plant life (Körner 2003) and may affect plant community assembly processes. Hutchinson demonstrated in (1957) that the region in which the multidimensional space of environmental factors positively affects population growth is defined as the population niche. Later, Grubb (1977) considered the species' niche with four components: the habitat, the lifeform, and the regeneration niche. The regeneration niche is mainly modelled by the physical and chemical requirements for species' survival and reproduction and it has been considered to be important for determining plant community properties including stability and change (Donohue *et al.* 2010; Burghardt *et al.* 2015).

Germination is an irreversible process regulated by many abiotic factors such as temperature, soil moisture and light (Fenner and Thompson 2005). Therefore, the environmental conditions in which a seed germinates will influence a whole plant's lifecycle. Differences in germination

behavior among and within related taxa have been attributed to habitat type (Giménez-Benavides *et al.* 2007; Baskin and Baskin 2014) suggesting that natural selection exerts pressure on germination phenology. For example, germination time determines flowering season in *Arabidopsis thaliana* (Wilczek *et al.* 2009). Furthermore, species cannot persist in an ecological community if environmental conditions are only suitable for plant growth and survival, but not for seed germination (Poschlod *et al.* 2013). Germination traits are expected to play an important role in filtering the regional species pools into local communities (Jiménez-Alfaro *et al.* 2016). Seed germination traits are beginning to gain more attention (Orru *et al.* 2012; Porceddu *et al.* 2013; De Vitis *et al.* 2014; Fernández-Pascual *et al.* 2015; Dürr *et al.* 2015; Ordoñez-Salanueva *et al.* 2015; Bastos *et al.* 2017; Seal *et al.* 2017) and are now accepted as requiring better integration in plant community ecology (Jiménez-Alfaro *et al.* 2016; Larson and Funk 2016). These studies show that germination indices, e.g., using thermal (Bradford 2002) and hydro time models (Hardegree *et al.* 2013), can provide powerful information relating to the germination responses to temperature and soil moisture (Huang *et al.* 2016).

In this study, we examine a selection of species from siliceous and calcareous alpine grassland habitats, which are the two-main high altitude alpine habitats across temperate Europe. We selected seven characteristic species from each habitat and compared them in terms of their germination and vegetation traits. Traits selected for this study related to the germination niche include the three cardinal temperatures for germination rate (base, optimal and ceiling temperature), the base water potential for germination rate and total germination pH range. Vegetative traits selected for this study included leaf area (LA), specific leaf area (SLA), leaf dry matter content (LDMC), obtained by querying TRY-database. The climate of both case study habitats is similar, but differ in soil, and moisture holding capacity (Körner 2003). Consequently, alpine calcareous soils are characterized by small soil grains (Franz 1979, *in Körner 2003*), drier conditions (Körner 2003; Hofmann *et al.* 2016) and have higher pH than siliceous alpine habitats (Hofmann, 2016). We asked if niche conditions may favour specific species strategies, thereby contributing to the environmental filtering of the species pool. We hypothesized that: 1) vegetative traits differ between habitats; and 2) there are habitat-related regeneration (germination) strategies related to specific environmental differences between sites. Particularly, we expect that species from calcareous habitats may germinate better under lower water potential than those from siliceous habitats, while siliceous species may germinate better under lower pH levels.

3.2 Materials and methods

3.2.1 Study system

Target species were collected from Site of Community Interest (SCI) “Passo dello Stelvio (46° 32’N, 10° 25’E)”, located in the Alps of Lombardy (Sondrio, Northern Italy). This SCI belongs to two habitats types defined by the European Habitat Directive Natura 2000 classification: Habitat #6230- Nardus-rich species grasslands’ (referred to as “siliceous habitat” throughout); and Habitat #6170- Alpine calcareous grasslands’ (referred to as “calcareous habitat” throughout) (European commission, 2007). These habitats were chosen because they represent two main vegetation types of the European high alpine zone, and are habitats of conservation concern. Different rock types, give rise to different soil acidities and properties. These factors seem to be important to plant species and vegetation type distributions (Nagy and Graber, 2009). However, the factors affecting species occurrence in alpine soils are still not fully understood.

3.2.2 Species selection and seed collection

Six congeneric species were selected from each habitat, specifically *Achillea moschata* Wulfen and *Achillea nana* L., *Campanula barbata* L. and *Campanula cochleariifolia* Lam., *Festuca halleri* All. and *Festuca pumila* Chaix, *Gentiana acaulis* L. and *Gentiana clusii* E.P. Perrier & Songeon, *Phyteuma hemysphaericum* L. and *Phyteuma orbiculare* L., *Pulsatilla vernalis* L. (Mill) and *Pulsatilla alpina* L. In addition, one pair of species from the same taxonomic family (*Geum montanum* L. and *Dryas octopetala* L.) was chosen. Species were selected and collected based on the following criteria: seed availability, habitat provenance (i.e., presence in either #6230 or #6170) and abundance of individuals at the collection site. Freshly matured seeds were harvested from 150-200 plants of each species at the time of natural dispersal in 2016 (i.e. August to October). Finally, a database with species’ leaf area (LA), specific leaf area (SLA) and leaf dry matter content (LDMC) was built by querying TRY-database (Kattge *et al.* 2011).

3.2.3 Germination experiments

After collection, seeds were cleaned, pooled and stored at room temperature until the beginning of the experiments, which occurred within 3 weeks after collection date. All germination experiments testing the effect of temperature, drought and pH were carried out sowing three replicates of 20 seeds each, using temperature and light controlled incubators (LMS 250A, LMS Ltd, Sevenoaks, UK) at 12-h daily photoperiod (photosynthetically active radiation 40 – 50 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Plates were checked for radicle emergence (>2mm) daily until germination

was stopped (i.e. no emergence over 7 days period). At the end of the experiments, non-germinated seeds were cut-tested to check their viability, with soft seeds being counted as inviable. Then, the final germination percentage (FGP) and the cumulative germination was calculated excluding non-viable seeds.

The effect of temperature was tested on both fresh seed and seeds treated with 250 mg/l of gibberellic acid (GA₃) (referred to hereafter as “untreated” and “GA₃-treated” seeds, respectively) and sown in agar. The effects of pH and drought were investigated only on seeds that had been cold (wet) treated for three months at 0 °C in complete darkness (referred to hereafter as “cold-treated”). After cold stratification, seeds were dried (15%RH, 15°C), sown in paper and placed in germination incubators under the various conditions. Cold stratification and GA₃ were chosen as treatments as they were previously found to effectively release dormancy on the target species (Tudela-Isanta et al. 2017).

To estimate the cardinal temperatures for germination rate, GA₃-treated and untreated seeds were incubated under different daily constant temperatures ranging from 0 to 40°C, with increases of 5°C between each treatment. The effect of drought on germination was assessed incubating cold-treated seeds under nine different water potential conditions (i.e. 0, -0.1, -0.2, -0.4, -0.6, -0.8, -1, -1.5, -3 MPa) at 25/15°C. The desired water potential was obtained using different concentrations of polyethylene glycol (PEG) 8000 (Amresco, LLC), in accordance with Michel (1983), with the higher temperature (i.e., 25°C) taken into consideration as a reference. Finally, the effect of pH was tested on cold-treated seeds at 25/15°C under different pH regimes ranging from 4.5 to 7.5, by adjustment using different concentrations of Hydrochloric acid (HCl) and Sodium hydroxide (NaOH) in acetic-acetate buffer.

3.2.4 Calculation of germination traits

The cumulative germination percentages (30%, 50% and 60%) were plotted against time using the equation with the best fit (i.e. log-logarithm) to calculate the T₃₀, T₅₀ and T₆₀. Cardinal temperature points including base, optimal and ceiling temperatures (referred to hereafter by T_b, T_o and T_c, respectively) for germination rate were estimated using thermal models (García-Huidobro et al. 1982) and base water potential for germination rate (referred to hereafter by Ψ_b) was estimated using hydromodels (see Gummerson 1986). The thermal models and hydro models were fit using the inverse of T₃₀, T₅₀ and T₆₀ and regressed against temperature and water potential, respectively. T₃₀ was chosen as the measurement threshold because the target species are known to be dormant at dispersal, hence untreated seeds do not reach high

germination scores; T_{50} and T_{60} were chosen to include a higher representation of the population. When there was insufficient germination to enable a reliable estimate of the cardinal temperatures, lower and upper thresholds were assigned to the temperature closest to the one at which germination at zero. If germination was only scored in one temperature this temperature was considered as being the T_o .

3.2.5 Statistical analysis.

All statistical analyses and plotting were done using the R Studio version 3.2.2. To test vegetative traits between habitats three different one-way-ANOVAs were used. When the response variable was not normally distributed, data was log transformed. Next, germination traits (i.e., cardinal temperatures and Ψ_b) estimated with T_{30} , T_{50} and T_{60} were compared using a one-way and two-way ANOVA analyses were performed to test differences between germination traits (T_b , T_o , T_c), habitat and treatment; ONE-WAY ANOVA analyses were carried out to test if there were differences between habitats and Ψ_b . Because Ψ_b was not normally distributed a square transformation was applied before proceeding with the statistical analysis.

The pH results were analysed using Generalized Linear Mixed Models (GLMM) with logit link function and binomial error structure or quasi-binomial error structure, when residual over-dispersion was present. The seed germination proportion (i.e. number of emerged radicles out of the number of viable seeds of each species) was the response variable, whereas, pH [4-level categorical variable including 4.5, 5.5, 6.5 and 7.5], habitat (2 level categorical variable, including siliceous and calcareous), and their interaction were the explanatory variables. Finally, species (14-level categorical variable) were treated as a co-variable. The model with the best fit was selected using Akaike's Information Criterion (AIC) (Burnham *et al.* 2011). In addition, GLMs for each species and treatment were used to test the effect of pH at the species' level. In this model, FGP was the response variable and pH [4-level categorical variable including 4.5, 5.5, 6.5 and 7.5] was the explanatory variable.

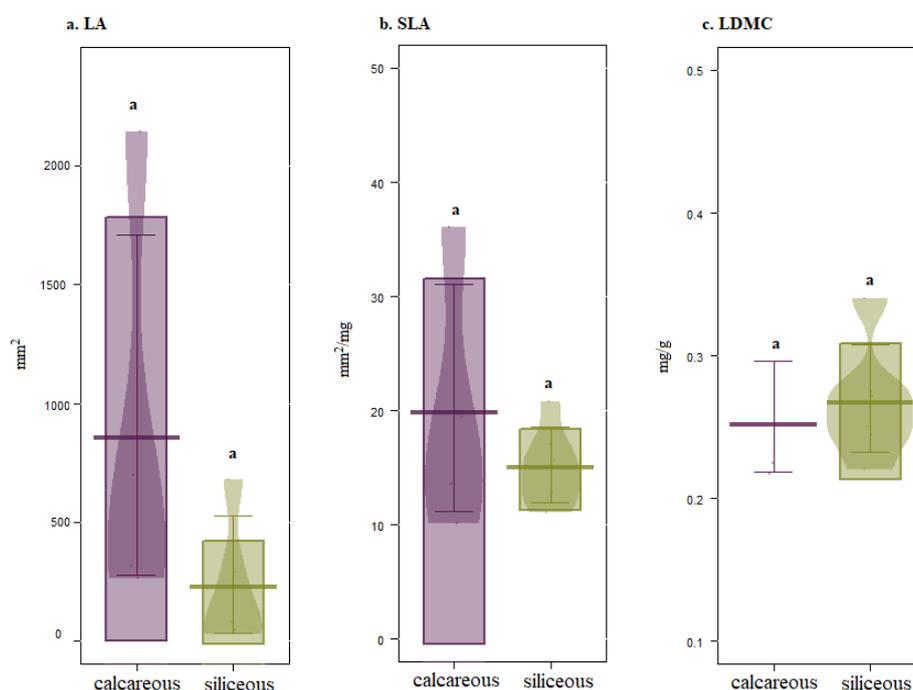
Finally, a Principal Component Analysis (PCA) using the "FactoMineR" package (Lê *et al.* 2008) was used to assess which traits explain species' habitat provenance better. In the first PCA, the three vegetative traits were included (i.e. SLA, LA and LDMC); the second included seven species' germination traits (i.e., FGP at 4.5, 5.5, 6.5 and 7.5 for cold-treated seeds; T_b for GA₃-treated and untreated; Ψ_b for cold- treated).

3.3 Results

3.3.1 Vegetative traits.

The trait data was compiled from pre-existing datasets contributing to the TRY Plant Trait Database (see Supplementary material 3.1). SLA values ranged from $36.1 \text{ mm}^2 \text{ mg}^{-1}$ (i.e. *Phyteuma orbiculare*) to $10.09 \text{ mm}^2 \text{ mg}^{-1}$ (i.e. *Gentiana acaulis*), with a mean of $16.83 \text{ mm}^2 \text{ mg}^{-1}$. LA for the target species varied from 2140.56 mm^2 (i.e., *Pulsatilla alpina*) to 25.24 mm^2 (i.e., *Festuca halleri*), with a mean of 513.42 mm^2 . Finally, the value of LDMC ranged from 0.2 mg g^{-1} to 0.34 mg g^{-1} , with a mean of 0.24 mg g^{-1} . High variability within habitats was detected for all vegetative traits. Number of species included in the analysis were 11 for SLA, 10 for LA and nine for LDMC because data was not always available. Differences in SLA, LA or LDMC are not a result ($P = 0.08, 0.444$ and 0.61 , respectively) of species' habitat (Figure 3.1 a, b and c).

Figure 3.1. RDI plots including (Raw data, Descriptive and Inference statistics) show jittered points of raw data, center point indicates mean vegetative traits from the left to the right LA (a), SLA (b) and LDMC (c). Beans outline the smoothed density of the data, whiskers mark the 10% and 90% quartiles of the data, and inference bands show the Bayesian 95% High Density Interval inferential statistic for each habitat. Green RDI plots representing seeds with siliceous provenance and in purple species inhabiting in the calcareous bedrock.

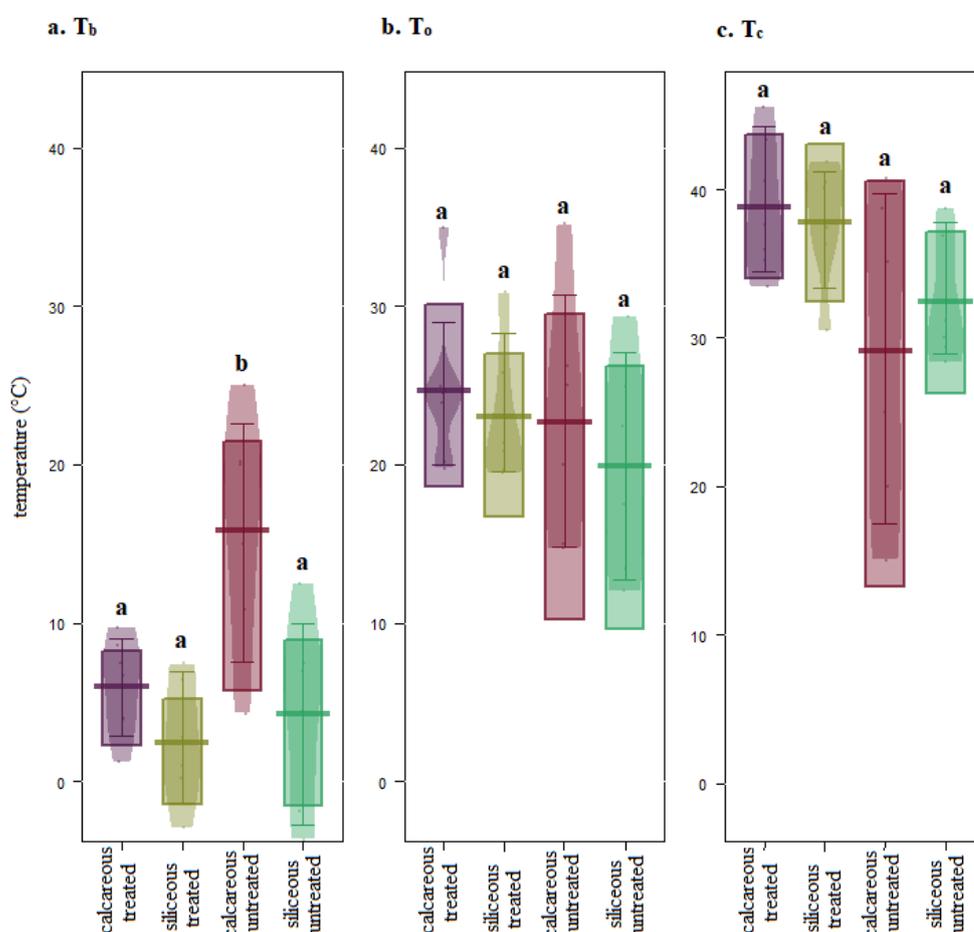


3.3.2 Cardinal temperatures

Overall, GA₃-treated and untreated seeds produced very different cardinal temperatures (Table 3. 1). No significant differences were detected between the cardinal points estimated with T₃₀, T₅₀ and T₆₀, (i.e. $P > 0.05$) (see Supplementary material 3. 2 for cardinal temperature

values estimated with T_{50} and T_{60} rates). Consequently, the cardinal temperatures estimated with the T_{30} rates were used for further analysis and are the ones reported below. This permitted the untreated seed of more species to be included in the analyses. Because some species are dormant when dispersed FGP did not always reach 50% of germination. The estimation of cardinal temperatures was not possible for one species (*Geum montanum*), even for GA₃-treated seeds because of a fungus infection and for two species (*Gentiana acaulis* and *Gentiana clusii*) as no germination was recorded for the untreated seeds.

Figure 3.2. RDI plots including (Raw data, Descriptive and Inference statistics) show jittered points of raw data, center point indicates mean temperatures of germination from the left to the right T_b , T_o and T_c for the target species. Beans outline the smoothed density of the data, whiskers mark the 10% and 90% quartiles of the data, and inference bands show the Bayesian 95% High Density Interval inferential statistic for each habitat. Green RDI plots representing GA₃-treated and untreated seeds with siliceous provenance and in purple species inhabiting in the calcareous bedrock.



The TWO-WAY ANOVA analysis revealed that the T_b values were significantly lower ($P = 0.01407$) for GA₃-treated seeds in comparison with untreated seeds (Figure 3.2a). In contrast, the values of T_c increased significantly ($P = 0.00597$) for GA₃-treated seeds (Figure 3.2c). Though, T_{op} for GA₃-treated and untreated seeds (Figure 3.2b) ($P=0.30$) was similar. Also, species from siliceous habitats had significantly ($P= 0.00443$) lower T_b values than species

from calcareous soils (Figure 3.2a). However, T_o and T_c (Figure 3.2b and 3.2c) were not affected by habitat provenance ($P=0.35$ and 0.762 respectively).

3.3.3 Water potential

No significant differences ($P > 0.05$) were detected between the estimated Ψ_b and the rates (T_{30} , T_{50} and T_{60}) (see Supplementary material 3.3 for Ψ_b estimated with T_{50} and T_{60} values). The values of Ψ_b ranged from -0.39 (i.e. *Geum montanum*) to -0.91 (i.e. *Festuca pumila*), with a mean of -0.6 MPa (Table 3.2). The ONE-WAY ANOVA revealed that species from calcareous habitats had significantly lower Ψ_b ($P = 0.0293$) than their siliceous, congeneric species (Figure 3.3). Ψ_b was not estimated for *Campanula barbata* because the seed supply was inadequate.

Figure 3.3 RDI plots including (Raw data, Descriptive and Inference statistics) show jittered points of raw data, center point indicates mean Ψ_b of germination. Beans outline the smoothed density of the data, whiskers mark the 10% and 90% quartiles of the data, and inference bands show the Bayesian 95% High Density Interval inferential statistic for each habitat. Green RDI plots representing cold-treated seeds with siliceous provenance and in purple species inhabiting in the calcareous bedrock.

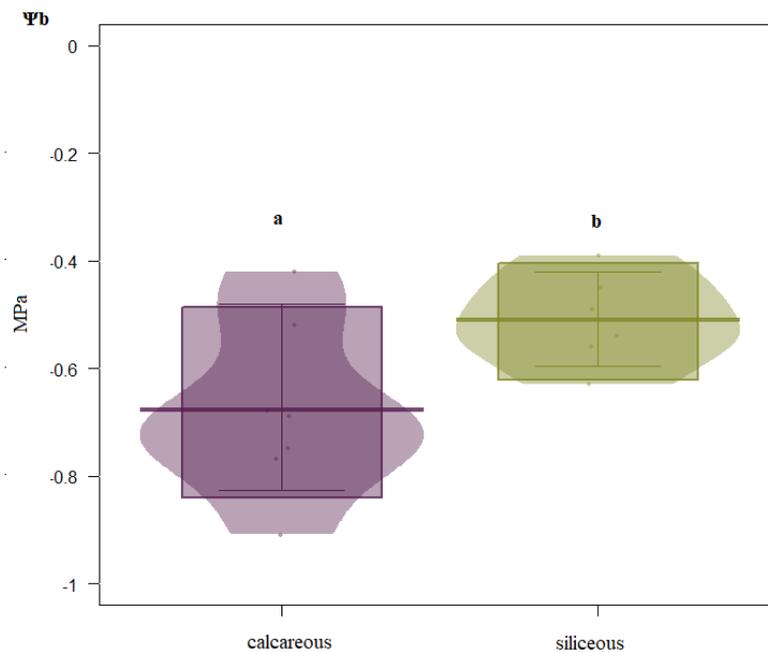


Table 3.1. Mean estimated T_b , T_o , and T_c for each species resulting from the fitted thermal models.

Specie	Habitat	GA ₃ -treated seeds					Untreated seeds				
		T_b	T_o	T_c	R^2_{Sbop}	R^2_{Spop}	T_b	T_o	T_c	R^2_{Sbop}	R^2_{Spop}
<i>Achillea moschata</i>	Siliceous	-2.96	30.89	41.89	0.90	0.73	12.48	29.34	38.75	0.88	0.50
<i>Achillea nana</i>	Calcareous	8.58	34.96	40.56	0.88	0.98	20.14	35.22	40.71	0.88	0.68
<i>Campanula barbata</i>	Siliceous	2.79	25.76	40.51	0.92	0.94	6.90	22.40	31.17	0.69	0.72
<i>Campanula cochlearifolia</i>	Calcareous	4.01	24.96	37.65	0.98	0.97	20	20	20	NA	NA
<i>Festuca halleri</i>	Siliceous	0.22	19.46	40.05	0.77	0.76	4.45	13.40	29.42	0.93	0.88
<i>Festuca pumila</i>	Calcareous	1.21	24.49	35.20	0.93	0.99	4.27	14.68	38.69	0.88	0.99
<i>Gentiana acaulis</i>	Siliceous	6.42	21.37	36.30	0.99	0.88	NA	NA	NA	NA	NA
<i>Gentiana clusii</i>	Calcareous	7.49	19.74	35.97	0.76	0.79	NA	NA	NA	NA	NA
<i>Phyteuma hemysphaericum</i>	Siliceous	0.98	20.82	37.38	0.84	0.84	-3.67	12.01	28.38	0.59	0.88
<i>Phyteuma orbiculare</i>	Calcareous	3.94	20.13	45.58	0.92	0.59	15	15	15	NA	NA
<i>Pulsatilla vernalis</i>	Siliceous	7.43	19.68	30.47	0.98	0.98	7.44	24.89	36.87	0.98	0.96
<i>Pulsatilla alpina</i>	Calcareous	6.71	23.91	33.43	0.97	0.54	25	25	25	NA	NA
<i>Geum montanum</i>	Siliceous	NA	NA	NA	NA	NA	-1.875	17.47	30	0.92	0.59
<i>Dryas octopetala</i>	Calcareous	9.7	24.64	43.28	0.99	0.65	10.87	26.18	35.10	0.94	0.60

R^2_{Sbop} and R^2_{Spop} . Estimated r squared from the regression lines from T_{30} rates against temperature from the suboptimal and supraoptimal respectively.

T_b , T_o and T_c . Base, optimal and ceiling temperature for germination

Table 3.2. Mean estimated Ψ_b for each species resulting from fitted hydro models.

Specie	Habitat	Cold-treated seeds	
		Ψ_b	R^2
<i>Achillea moschata</i>	Siliceous	-0.45	0.97
<i>Achillea nana</i>	Calcareous	-0.42	0.98
<i>Campanula barbata</i>	Siliceous	NA	NA
<i>Campanula cochleariifolia</i>	Calcareous	-0.69	0.90
<i>Festuca halleri</i>	Siliceous	-0.49	0.49
<i>Festuca pumila</i>	Calcareous	-0.91	0.99
<i>Gentiana acaulis</i>	Siliceous	-0.54	0.99
<i>Gentiana clusii</i>	Calcareous	-0.77	0.99
<i>Phyteuma hemysphaericum</i>	Siliceous	-0.63	0.98
<i>Phyteuma orbiculare</i>	Calcareous	-0.75	0.93
<i>Pulsatilla vernalis</i>	Siliceous	-0.56	0.97
<i>Pulsatilla alpina</i>	Calcareous	-0.68	0.86
<i>Geum montanum</i>	Siliceous	-0.39	0.97
<i>Dryas octopetala</i>	Calcareous	-0.52	0.65

R^2 . Estimated r squared from the regression lines from T_{30} rates against water potential.

Ψ_b : base water potential.

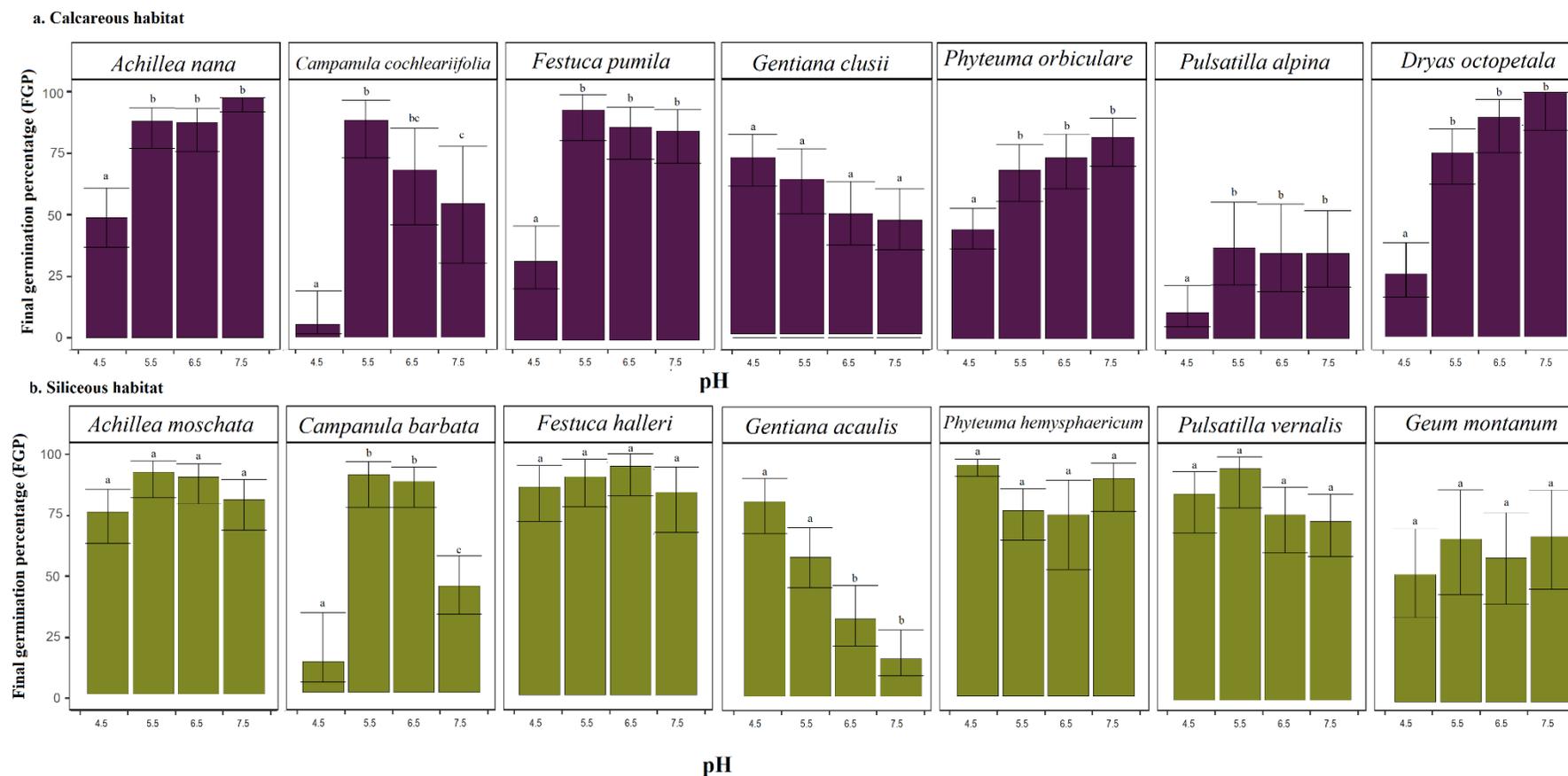
3.3.4 pH

Overall, total seed germination among species was affected by the different pH regimes (Table 3.3). FGP values ranging from 5% (i.e., *Campanula cochleariifolia*) to 100% (i.e., *Pulsatilla vernalis* and *Achillea nana*) were recorded along the pH gradient. The mean FGP values were 47.1%, 78.3%, 73.0% and 68.0% for 4.5, 5.5, 6.5 and 7.5 pH, respectively. The pH had a significant effect on FGP for eight out of the 14 tested species. Five species (i.e., *Achillea nana*, *Dryas octopetala*, *Festuca pumila*, *Phyteuma orbiculare* and *Pulsatilla alpina*) had a significantly lower FGP when seeds were sown at pH 4.5. *Campanula cochleariifolia* and *Campanula barbata* had their optimum FGP at pH 5.5 and 6.5, and a significantly lower FGP was recorded when seeds were incubated at pH 7.5 and 4.5. Finally, *Gentiana acaulis* had significantly higher FGP when seeds were sown under acidic conditions (pH 4.5 and 5.5).

Table 3.3 Mean final germination percentage (FGP) for each species under the different pH (\bar{x}), including their lower (LW) and upper (UP) limits. Significant differences among treatments within each species are indicated by different letters.

Species treated	Habitat	4.5			5.5			6.5			7.5		
		\bar{x}	LW	UP	\bar{x}	LW	UP	\bar{x}	LW	UP	\bar{x}	LW	UP
<i>Achillea moschata</i>	Siliceous	75.93 ^a	62.83	85.48	92.59 ^a	81.87	97.19	90.57 ^a	79.26	96.02	81.13 ^a	68.36	89.54
<i>Achillea nana</i>	Calcareous	50.00 ^a	37.61	62.39	90.38 ^b	78.90	95.94	89.80 ^b	77.73	95.69	100.00 ^b	94.13	100.00
<i>Campanula barbata</i>	Siliceous	13.04 ^a	4.27	33.55	92.11 ^b	78.20	97.43	89.29 ^b	78.13	95.11	45.00 ^c	32.98	57.64
<i>Campanula cochleariifolia</i>	Calcareous	5.12 ^a	1.28	18.31	86.49 ^b	71.38	94.26	70.00 ^{bc}	51.66	83.59	57.14 ^c	38.68	73.81
<i>Festuca halleri</i>	Siliceous	82.61 ^a	68.91	91.06	86.79 ^a	74.79	93.57	91.84 ^a	80.18	96.90	80.56 ^a	64.47	90.44
<i>Festuca pumila</i>	Calcareous	30.77 ^a	19.78	44.47	89.80 ^b	77.73	95.69	83.02 ^b	70.47	90.92	81.48 ^b	68.89	89.74
<i>Gentiana acaulis</i>	Siliceous	77.77 ^a	64.82	86.92	55.55 ^a	43.19	67.26	30.90 ^b	20.16	44.22	15.00 ^b	7.99	26.38
<i>Gentiana clusii</i>	Calcareous	72.05 ^a	60.29	81.41	63.26 ^a	49.07	75.48	49.09 ^a	36.24	62.06	46.55 ^a	34.20	59.33
<i>Phyteuma hemysphaericum</i>	Siliceous	95.05 ^a	90.59	97.77	76.67 ^a	64.37	85.67	81.60 ^a	68.00	88.28	90.00 ^a	76.21	96.20
<i>Phyteuma orbiculare</i>	Calcareous	44.36 ^a	36.15	52.88	68.33 ^b	55.61	78.80	73.33 ^b	60.81	82.97	81.67 ^b	69.85	89.55
<i>Pulsatilla vernalis</i>	Siliceous	100.00 ^a	63.06	100.00	93.55 ^a	77.58	98.38	75.00 ^a	59.46	85.99	72.34 ^a	57.99	83.21
<i>Pulsatilla alpina</i>	Calcareous	10.00 ^a	4.56	20.53	31.25 ^b	19.80	45.56	38.46 ^b	24.69	54.37	42.80 ^b	20.00	60.00
<i>Geum montanum</i>	Siliceous	58.33 ^a	38.34	75.91	58.33 ^a	38.34	75.51	51.51 ^a	34.93	67.77	59.26 ^a	40.30	75.81
<i>Dryas octopetala</i>	Calcareous	25.00 ^a	15.67	37.42	73.33 ^b	60.81	82.97	87.50 ^b	73.28	94.70	67.50 ^b	51.73	80.10

Figure 3.4. Final germination percentage (FGP) and confidence interval for species under different pH regimes. The top set of graphs, with purple bars, represents species from calcareous habitats, while the bottom set of graphs, with green bars, represents species from siliceous habitats.



The GLMM (AIC= 763.0) of FGP was explained best by habitat and pH, including species as a co-variable. This indicates that habitat and pH had a significant effect on the FGP (Supplementary material 3.4). Siliceous species (Figure 3.4b) had a significantly higher FGP when seeds were incubated at pH 4.5 than calcareous species (i.e. 61.9% vs 32.4%, respectively) (Figure 3.4a).

3.3.5 Trait-habitat clusters.

Germination and vegetative traits were analysed separately in the multivariate space using two separate PCAs. In the vegetative traits-PCA the total of variance explained by the two-principal axes was 83.72% (See Supplementary Material 3.5). Axis I (representing 50.94% of the total variance) was mainly explained by LDMC and the Axis II (representing 32.78% of the total variance) by LA. When species were clustered by habitat, the 95% confidence level ellipses demonstrate little difference, indicating that species' inter-habitat variability is not higher than species intra-habitat variability for these traits (Figure 3.5a). In the germination traits-PCA (See Supplementary Material 3.6) the two-principal axes explained 65.98% of the variance. T_b of GA₃-treated and untreated seeds and the FGP at pH 5.5, 6.5 and 7.5 contributed to the variation on Axis I (representing 41.90% of the total variance). The FGP at pH 4.5 and 7.5 contributed to the variation on Axis II (representing 22.66% of the total variance), where the species were strongly clustered according their habitat provenance. Most calcareous species were placed on the top-right part of the trait space, showing calcareous species have a higher T_b and prefer basic pH. Meanwhile species from the siliceous habitat are mostly placed on the lower-right part of the trait-space, indicating the preference of acidic pH and lower T_b (Figure 3.5b).

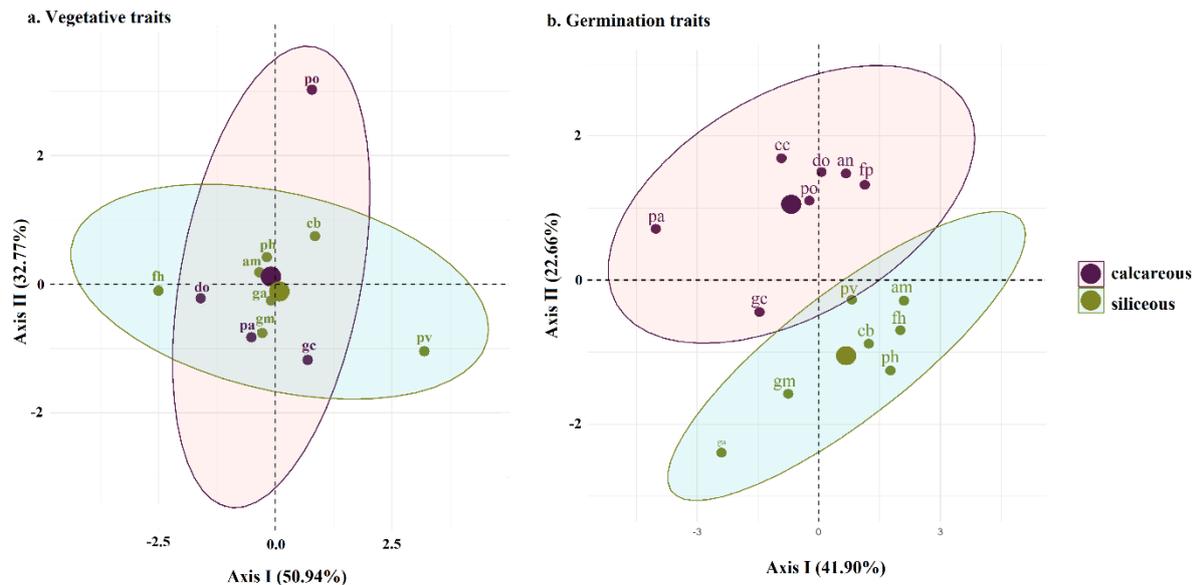
3.4 Discussion

Overall, our results indicated differences within and between habitats when considering both germination and vegetative traits. Vegetative traits were similar between habitats hence we rejected hypothesis one. Meanwhile, specific habitat-regenerative strategies were identified for some regenerative traits, partially accepting hypothesis 2.

High variability between species was detected when considering both germination and vegetative traits. The specific germination requirements for alpine species has been reported previously (Körner 2003; Schwiendbacher *et al.* 2012; Orsenigo *et al.* 2015) and attributed to the short and highly variable growing season (Giménez-Benavides and Milla 2013) and to

taxonomic differences (Baskin and Baskin 2014). The interspecific variation in the vegetative traits examined here could be due to the life-form and taxonomic differences of the target species, which have been shown to be important factors causing divergence in traits values when exploring global plant variability (Díaz *et al.* 2015; Pierce *et al.* 2017).

Figure 3.5. Principal component analysis using species' vegetative traits (a) and species' germination traits (b). Species were clustered by habitat provenance representing in purple species inhabiting in calcareous habitats and in green species inhabiting in siliceous habitats.



Importantly, our results show that germination traits contribute to the separation of species by their habitat in the multivariate space according to their habitat, while habitat clusters based on vegetative traits were very similar (Figure 3.5a and 3.5b). The convergence of vegetative traits shown here suggests a narrower, no habitat trait differentiation among adult plant species attributed to the high species variability existing within habitats. The separate habitat clusters identified when using germination traits (Figure 3.5b) suggests that germination traits and niche may limit species occurrence before the adult stage (McKee and Richards 1996). Interestingly, species from calcareous habitat showed higher T_b , low Ψ_b and low FGP under acidic pH than closely related species from siliceous habitats, indicating the presence of different germination niches in the two habitats. Conversely, vegetative traits (SLA, LA and LDMC) and some other germination traits (i.e. T_o , T_c) were not different between habitats. Vegetative traits contribute to explain the variability of plant species globally (Wright *et al.* 2004; Poorter *et al.* 2008; Nguyen *et al.* 2014; Díaz *et al.* 2015; Pierce *et al.* 2017), though they are not always fundamental for explaining local plant population dynamics (Larson and Funk 2016). Importantly, the higher habitat vegetative traits

convergence and the specific habitat-related germination niche detected here, strongly suggest that species occurrences in calcareous and siliceous habitats may be filtered by germination constraints. This is a novel and interesting observation highlighting that the germination niche is a key aspect of the regeneration niche (Grubb 1977), for setting community trajectories and driving ecosystem function and services (Fraaije *et al.* 2015).

Grubb (1977) considered that the regeneration niche is mainly affected by species' chemical and physical requirements for survival. In this case, dissimilarities in the T_b between calcareous (6.02°C) and siliceous (2.48°C) habitats may indicate the existence of some environmental filtering driving the divergent evolution of specific habitat-related regenerative strategies. Although air temperatures may be similar in both habitats (i.e., often located in adjacent areas), soil temperatures may differ (Graham *et al.* 2012). Topsoil desiccation in summer is common in calcareous bedrocks (Kammer and Mohl 2002) and drier soils are also warmer because of their poor thermal conductivity (Graham *et al.* 2010). Consequently, species inhabiting warmer soils have higher T_b , as they have adapted to need more heat as a cue to initiate germination. Because soil temperatures in calcareous habitats may be higher, the possibility that species need a higher T_b to prevent germination at dispersal time, thereby avoiding seedling mortality due to hyperthermia in summer or frost events in winter (Rosbakh and Poschlod 2015; Fernández-Pascual *et al.* 2017) is also possible.

Calcareous species also showed lower Ψ_b than siliceous, suggesting that species from calcareous bedrocks can germinate when there is lower water availability. This is consistent with previous findings that soil moisture is a limiting factor in plant communities typically inhabiting calcareous bedrocks (Kammer and Mohl 2002). Hence, lower Ψ_b on calcareous bedrocks suggests these species seeds have the ability to germinate under smaller rainfall events, which may have long-term benefits at the community level. Similarly, in other water restricted habitats like the Desert Laboratory at Tumamoc Hill in Arizona, species with short thermal times and low Ψ_b have been considered as high-integrated-water-use efficient and tend to be more successful under drought conditions than species with higher Ψ_b and hydro time (Huang *et al.* 2016).

Meanwhile in siliceous habitats, species rarely suffer water restrictions, hence species are not specifically adapted to germinate at low water potentials and showed higher Ψ_b . Moreover, siliceous' species had high FGP scores under acidic pH treatments. Only one species out of seven (i.e. *Campanula barbata*) had significantly reduced FGP at pH 4.5 compared with the other pH regimes. In contrast, in the calcareous habitat five out of seven species had

significantly decreased FGP when exposed to acidic treatments. The acidity of siliceous bedrocks increases aluminium solubility in the soil (Clarkson 1969) and higher tolerance to aluminium concentrations have been detected in seedlings of species living in acidic soils than in seedlings of species from calcareous bedrocks (Abedi *et al.* 2013). The sensitivities of germination to pH support the hypothesis that species' adapt to survive in specific conditions of a habitat (Keddy 1992).

Finally, some common germination traits among habitats have been detected, such as high T_o and T_c and the treatment (i.e. GA3) effect on increasing the range of germination temperatures. The high T_o found in both habitats (i.e. 22.7 and 19.9°C, calcareous and siliceous respectively) may be a requirement for ensuring maximal seedling emergence rates without the risk of frost (Cavieres and Arroyo 2000; Fernández-Pascual *et al.* 2017). Indeed, higher T_o appears to be a common trait of alpine species compared with their congeneric species below the treeline (Walder and Erschbamer 2015). This germination strategy of alpine species is in accordance with our results, in which non-dormant seeds have a wider suitable temperature range for germination: i.e., lower T_b and higher T_c than untreated seeds. Most of the species studied here disperse dormant seeds, preventing germination in late summer (Fernández-Pascual *et al.* 2017). Indeed, physiological dormancy is common for alpine flora at the time of seed dispersal (Schwienbacher *et al.* 2011; Sommerville *et al.* 2013; Baskin and Baskin 2014). Dormancy loss triggers germination at favourable sites and times for seedling establishment (Fenner and Thompson 2005; Poschlod *et al.* 2013), and in alpine habitats, it is often after snowmelt in spring (Körner 2003; Schwienbacher *et al.* 2011). The likelihood of seedling recruitment is higher in spring because temperatures rise fast and there is lower risk of frost (Rosbakh and Poschlod 2015) and seedlings have the entire season to reach an optimal size for overwintering (Billings and Mooney 1968). High T_o , T_c and high T_b in untreated seeds, *Geum montanum* aside, must be common traits to successfully survive and establish in alpine habitats.

3.5 Conclusions

Analysing the germination niche of calcareous and siliceous bedrocks from alpine habitat, we provide new baseline data supporting the role of the germination niche as a primary filter to limit species distribution across habitats. The significant differences identified between germination traits in each habitat together with the germination traits in the multivariate space

indicate the existence of habitat-specialized-regenerative strategies and strongly suggests the importance of regenerative strategies on setting community trajectories in alpine habitats. Therefore, further studies are needed to investigate the extent to which the differences in germination traits found here reflect subsequent differences in emergence and survival in nature.

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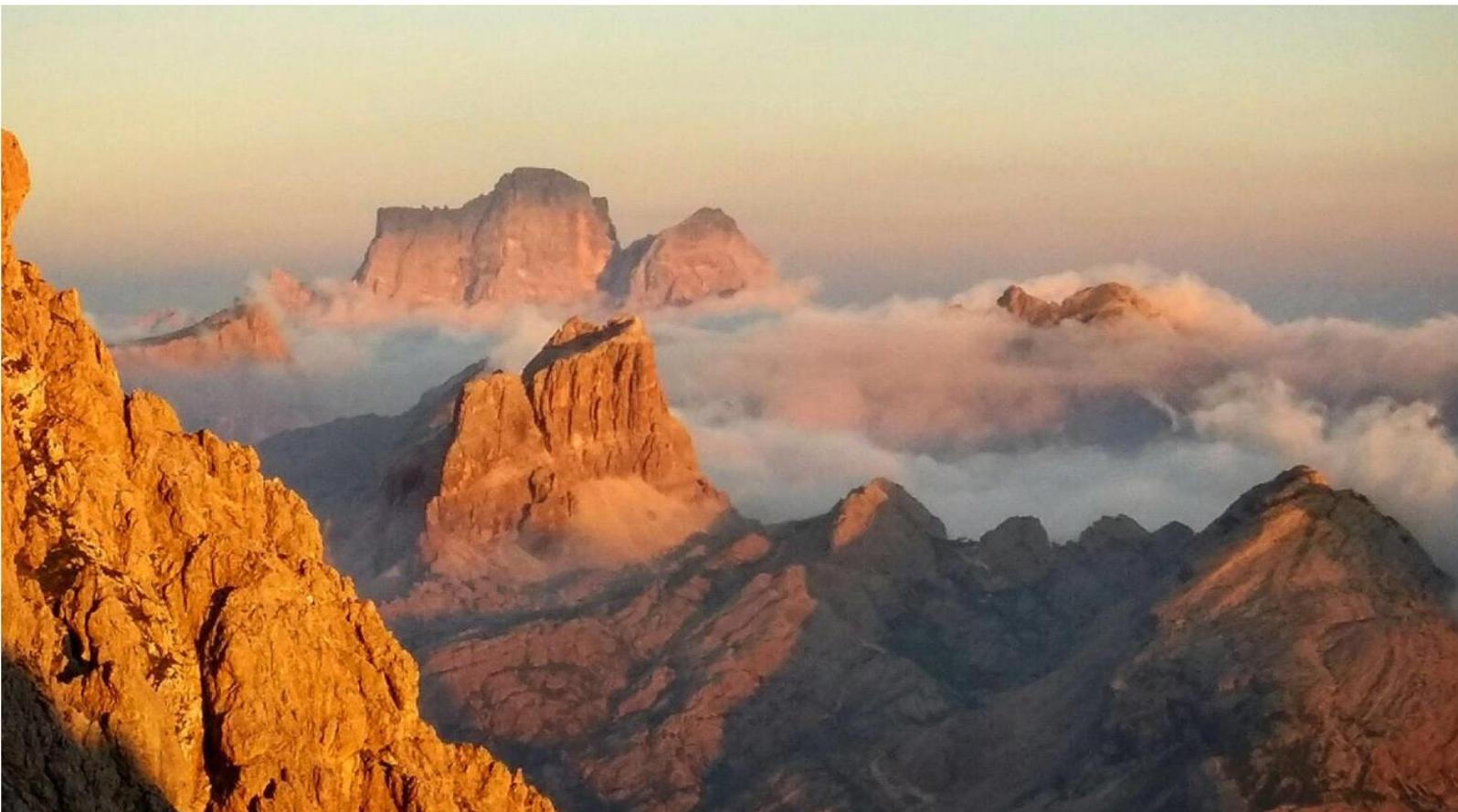
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Chapter 4

**Thermal germination models suggest distinct timing of emergence
in alpine plants inhabiting the same growing site**



Abstract

When and where seeds germinate set the context for plant development and natural selection. Seed thermal requirements are particularly important for regulating germination timing across habitats, thus thermal models on seed germination help in predicting plant regeneration under different climate scenarios. In alpine environments, different germination strategies have been observed across species from different habitats due to local environmental variability, while little is known about difference among co-existing species (i.e. sharing same growing site). However, the differentiation of germination niche and, in particular, of germination timing may have important implications on plant co-existence, reducing competition during recruitment. Here, we test potential differences in the germination timing of three perennial hemycrophytes living in acidic alpine grasslands of European Alps. We fit thermal models using germination test at different periods of cold stratification and soil heat sum, resulting from measurements at the species growing site to estimate the timing of seedling emergence. Results showed that base temperature and thermal time for germination decreased with increasing length of cold stratification in two species (*Achillea moschata* and *Festuca halleri*) and increased in one (*Pulsatilla vernalis*). Time of seed germination differed among all species and was predicted for 199, 254 and 351 days after dispersal. Species also showed distinct dormancy patterns. The different thermal thresholds and dormancy strategies detected here, suggests large differences on germination timing across species inhabiting in alpine grasslands. Regulating germination timing may allow co-existing species to reduce the probability of overlapping germination niches, thereby contributing to species' niche differentiation.

4.1 Introduction

Germination is an irreversible process in plant life. The place and time in which seeds germinate compromise seedling survival and set the context for plant development and natural selection (Donohue *et al.* 2010). Regeneration from seeds also play a key role in vegetation dynamics (Jiménez-Alfaro *et al.* 2016; Larson and Funk 2016). Germination cuing is strongly regulated by the environment (Fenner & Thompson 2005) and by the mother plant through genetic (Galloway & Fenster 2000) and epigenetic (Bernareggi *et al.* 2016) effects. The influence of climate is especially relevant to address germination patterns across latitudinal and altitudinal gradients, which is generally reflected in habitat-specific responses (Giménez-Benavides *et al.* 2007; Baskin and Baskin 2014).

Temperature and water are among the most important climatic drivers determining germination rate (i.e. speed of germination) and population germinability (Heydecker 1977). This is especially important in seasonal climates, where time of seed germination must occur when environmental conditions are favourable for seedling establishment (Grubb 1977; Poschlod *et al.* 2013) to increase the likelihood of recruitment. The importance of temperature on germination have led ecologists to develop mathematical functions called “thermal models”, which describe germination responses in function of temperature (García-Huidobro *et al.* 1982). These models assume an optimal temperature in which germination rates are the highest. Below this optimum, germination decreases progressively until a base temperature threshold (T_b) below which germination rate is zero. In addition, thermal time (θ) defines the degree-days above T_b required by a proportion of the population to germinate. Pritchard (*et al.* 1999) described a gradual reduction of T_b with time of stratification for dormant seeds of *Aesculus hippocastanum*, thus allowing germination to occur at lower temperatures. This information has been useful for agronomic applications to predict suitable moment for weed managing or appropriate timing of crop sowing (Grundy *et al.* 2003; Batlla & Benech-Arnold 2004). Recently, thermal models have been also applied to explore species germination under climate warming scenarios (Orrù *et al.* 2012; Porceddu *et al.* 2013; Fernández-Pascual *et al.* 2015), highlighting populations or species in which germination requirements will not be met.

Despite thermal time requirements are important for predicting germination behaviour under future climate scenarios, very little attention has been paid to wild species, particularly in alpine plants. Alpine habitats are characterized by harsh and long winters and high likelihood of frost events during autumn, where most seeds are generally conditionally dormant, i.e. they have a narrower window of germination temperatures than when they are not dormant (Baskin and Baskin 2014). In alpine environments, the suitable moment for germination is thought to be in spring, after snow melts (Körner 2003; Schwienbacher *et al.* 2011), when temperatures start to rise fast and there are lower chances of frost. Indeed, seeds from many alpine plants need high temperatures for germination (Billings & Mooney 1968; Walder & Erschbamer 2015). In general seeds are physiologically dormant at dispersal (Giménez-Benavides *et al.* 2005; Sommerville *et al.* 2013; Orsenigo *et al.* 2015) and dormancy is normally released with a period of cold stratification (Cavieres & Arroyo 2000; Schwienbacher *et al.* 2011; Hoyle *et al.* 2015). However, non-dormant species (Schwienbacher *et al.* 2011) or early germinators (Hoyle *et al.* 2015) also inhabit in alpine habitats, and a common alpine germination behaviour has not been identified yet. Indeed, different germination traits have been related to species occurrence along climatic (Rosbakh & Poschlod 2015) or altitude gradients (Vera 1997) in the alpine zone, and multiple germination alternatives seem to co-exist in similar habitats (Fernandez-Pascual *et al.* 2017, Tudela-Isanta *et al.* 2017) probably attributed to the large species pool and microhabitats existing in the alpine zone.

High elevation flora will be particularly sensitive to global warming due to their high specialization to low temperatures (Körner 2003; Inouye & Wielgolaski 2013; Carbognani *et al.* 2016) and the impossibility to migrate at higher altitudes because upwards migration is space limited (Engler *et al.* 2011). New climate scenarios may switch alpine germination timing from spring to autumn (Mondoni *et al.* 2012), or time of cold stratification may not be enough to overcome dormancy (Walck *et al.* 1997), reducing germination niches and consequently, threatening seedling recruitment. The ability of plants to survive under new climate scenarios will depend on their ecological niches (Gavin *et al.* 2014), hence it is related with the ecological niche range (Hampe & Petit 2005) and plant's adaptation competence (Crawford 2008). Seed germination can represent a major recruitment bottleneck in alpine populations, but we do not know to what extent germination niche and in particular, germination timing differed among

alpine species. Thus, the prediction of germination timing can provide an excellent tool to understand regeneration niches in alpine habitats, with implications in climate change assessment (Walck *et al.* 2011) and restoration (Merritt *et al.* 2011).

Here, we used thermal models to compare germination timing among three alpine species inhabiting at the same growing site in the European Alps. We chose three species plant specialists of acidic alpine grasslands that have shown different germination strategies in a previous study evaluating germination temperatures and dormancy (see Tudela-Isanta *et al.* 2017). Our main aim was to test whether these species also show differences in the regulation of dormancy that imply different times for germination. We collected fresh seeds in wild populations and conducted large germination experiments at different temperatures and periods of cold stratification; thermal models resulting from these test were compared with soil temperatures measured at the species growing sites to estimate accumulation of heat across time. Our specific aims were (1) to estimate thermal thresholds for predicting germination timing in the field, and (2) to compare germination phenology and seed dormancy in species from the same plant community.

4.2 Materials and methods

Study species and seed collection

Our target species are *Achillea erba rotta subsp moschata* Wulfen (Asteraceae) (refer here as *Achillea moschata*), *Festuca halleri* All (Poaceae) and *Pulsatilla vernalis* L (Mill) (Ranunculaceae). They are among frequent taxa that inhabit in alpine siliceous habitats of European Alps at around 2300 m a.s.l. (Aeschmann *et al.* 2004). This habitat occurs in nutrient-poor soils with acidic pH, formed on various types of siliceous rocks (mainly crystalline slides and granite, and volcanic rock).

Species were selected based on their habitat provenance, their germination strategy and seed availability at time of dispersal. Species are perennial hemicryptophyte that inhabits in alpine siliceous habitats above the treeline, though they have different biogeographical distribution range. *Achillea moschata* and *Festuca halleri* strictly occurs on alpine habitat, meanwhile *Pulsatilla vernalis* have a boreo-alpine distribution range inhabiting in alpine habitats but also in shrublands or roadsides (Zielińska *et al.* 2016). Target species showed different germination strategies: *Achillea moschata*

seems to germinate early in the spring, *Festuca halleri* showed rapid and high germination, without special germination restrictions and *Pulsatilla vernalis* probably germinates in late summer, since cold stratification decrease germination (Tudela-Isanta et al. 2017).

Seeds were collected at the time of natural dispersal on 30 of August 2015 in a Site of Community Interest (SCI) “Val Viola Dosde (46° 24’N, 10°12E’)”, located in the Alps of Lombardy (Sondrio, Italy) mainly dominated by the habitat 6230. At the sampling area a datalogger (Germini, Chichester, Uk) was buried 2 cm deep in the soil for an entire year (i.e. from September 2015 until October 2016) and soil temperature at hourly intervals was recorded. Datalogger was placed in the middle of the collection area, hence the furthest population was placed 800m in straight and 200m in altitude from the datalogger. Freshly mature seeds were collected from about 100-200 plants of the target species. From each plant around 20-100 seeds were collected depending on the availability of seeds. After collection, seeds were cleaned, pooled and stored at room temperature until the beginning of the experiments, which occurred maximum within 2 weeks after the collection.

Germination experiments

Seeds were placed in 1 % distilled water-agar in 50 mm diameter Petri dishes and were stratified at dark for 0, 30, 60, 90 and 150 days. After each interval plates were moved germination tests at five constant temperatures (i.e. 25, 20, 15, 10, 5 °C). For each species and treatment, three samples of 20 seeds each were used. Germination was scored when radicle protrusion was >15 mm (Pritchard et al. 1996) and it was checked daily until all seeds had germinated, or when no germination occurred for two consecutive weeks. The remained ungerminated seeds were cut-tested to confirm their viability. Seeds were considered non-viable when soft and/or necrotic tissues were observed.

Thermal models.

Thermal models were carried out for each period of cold stratification (i.e. 0, 30, 60, 90 and 150 days). The estimates of time ($t(g)$, days) from 5 percentiles of the population (i.e. 20, 30, 50, 65 and 80%, when germination was high, or 10, 20, 25, 35 and 50%, when germination was low) were interpolated from the cumulative germination curves fitted using Boltzmann equation. The inverse of the rate ($1/t(g)$) were regressed against

temperature using lineal models (Covell et al. 1986). The value of the intercept on the temperature axis below the optimal temperature for germination was considered as the T_b for each (g) percentile (Garcia-Huidobro et al. 1982). The average of the T_b among percentiles was calculated to establish the T_b of the population (Ellis 1986; Pritchard and Manger 1990) and linear regressions were recalculated for each percentile constraining to pass linear regressions through the new calculate population mean T_b . Thermal time for germination (θ_{sub}) was considered the slope of the suboptimal line of the refitted models (i.e. when lines were constrained to pass through the population T_b), hence the number of degrees above the T_b that seeds accumulate each day multiplied by the number of days reached since germination test started (t) (see equation 1).

$$\text{(Equation 1)} \quad 1/t_g \text{ (d}^{-1}\text{)} = (T - T_b) / \theta_g$$

Predicting models

The rate of change of T_b and θ_{50} by stratification period was calculated using linear regression, therefore T_b and θ_{50} were regressed against time of stratification. T_b and θ_{50} were normal or ln-normal distributed. The model showing a higher goodness of fit was selected based on the r^2 values (Hardgree, 2006). Differences on T_b and θ_{50} among treatments were assessed using Generalized Lineal Models (GLM) with a log link function and Poisson error structure. When negative values were present, square transformation was used to proceed with the analysis. Then, germination time in the field was predicted using a modified formula of environmental heat sum model (Orru et al. 2012) (equation 2). Environmental heat sum was calculated starting from May (i.e. after the period of cold stratification) for each species, but not for *Festuca halleri* because T_b was higher than cold stratification temperature, hence soil heat sum was accumulated during stratification period. Next, equation 2 was used to calculate species soil heat sum.

$$\text{(Equation 2)} \quad \text{Heat sum (}^\circ\text{C)/day} = \sum_{(1h, 2h...24h)} \{ [(T_{env} - T_b)] / 24 \},$$

in which, T_{env} is the average hour temperature recorded in the field and T_b is the base temperature calculated by day. Finally, species heat sum was compared with species thermal time to predict germination timing.

4.3 Results

Thermal models

Based on germination rate responses T_b varied among species and tended to decrease with cold stratification time (Table 1). The models in which T_b was fixed as the population mean T_b showed higher r^2 for all linear regression equations (Supplementary material 1) than the models where T_b varied for each percentile.

Achillea moschata T_b values (Table 1) decreased significantly (p .value<0.01) and the linear regression indicated that T_b decrease by 0.067°C per day of cold stratification (Figure 1). The θ_{50} decreased (i.e. $0.465\log^\circ\text{C day per log day}$ of cold stratification) significantly with length of cold stratification from 174.82° degrees-day at 0 days of cold stratification to 19.55 degrees-day (Figure 2). Time-log and θ_{50} -log normal distributed showed the best model fit. *Festuca halleri* T_b values (Table 1) decreased significantly with time of cold stratification (p .value<0.01) and the linear regression indicated that T_b decrease by 0.037°C per day of cold stratification (Figure 1). The θ_{50} decreased (i.e. $0.02\log^\circ\text{C day}^{-1}$) significantly, with an initial θ_{50} of 154.08 degrees-day to 7.23 degrees-day after 150 days of cold stratification (Figure 2). The best model was found when $\log \theta_{50}$ was regressed against normal time of cold stratification. T_b values of *Pulsatilla vernalis* (Table 1) were not significantly different among treatments (p .value= 0.14) (Figure 2). θ_{50} increase (i.e. 1.05 degrees-day) significantly with length of cold stratification from 97.56 at 0 days of cold stratification to 277.78 degrees-day after 150 days of cold stratification (Figure 2). Linear relation between linear θ_{50} and time of stratification showed the best fit.

Soil temperatures and soil heat sum models

The mean soil temperature was 4.49°C , the maximum temperature recorded was 44.24°C the 24th of June 2016 at 3 pm and the minimal temperature was -11.21°C recorded the 28th of November of 2015 at 7 and 8 am. In detail, the annual trend of soil temperature could be divided into five periods (i.e. P1, P2, P3, P4, P5) (Figure 3): P1 from the time of dispersal until mid-October were soil mean temperatures were above 5°C ; P2 from mid-October until the end of December were average soil temperatures were below zero and the snow cover was not thick enough to create thermal buffering hence with widen daily length temperature; P3 from January to the end of April were average soil temperature are below zero but snow cover is thick and daily length temperature narrow; P4 from May when snowmelt and temperature started increasing but mean average temperature were below 10°C and P5 from June to the end of

September 2016, temperatures increased and mean soil temperatures were generally above 10°C (Figure 3).

The soil heat sum was calculated according to the length of cold stratification recorded in the field, T_b decreasing rate (Figure 1) and equation 2. Subsequently, Σ soil heat sum was related with the species θ_{50} decreasing rate (Figure 3). Soil data showed the effective length of stratification periods was 206 days (i.e. P2 and P3), leading thermal time after cold stratification to 15.62, 2.4, 325.71 degrees-days and T_b to -1.98, -3.03°C, 8.75°C for *Achillea moschata*, *Festuca halleri* and *Pulsatilla vernalis*, respectively. However, *Achillea moschata* is known to not germinate below 0°C (*unpublished data*), therefore, we corrected the model fixing the lowest T_b at 0.6°C (corresponding to the 150 days of cold stratification). Conversely, *Pulsatilla vernalis* T_b remained unchanged after cold stratification. Consequently, soil heat models predicted germination timing 199 (March 3rd), 254 (May 11th) and 321 (July 18th) days after dispersal for *Festuca halleri*, *Achillea moschata* and *Pulsatilla vernalis*, respectively (Figure 3).

4.4 Discussion

In alpine habitats, germination timing has been suggested to be strongly controlled by temperature (Rosbakh & Poschlod 2015), but very few studies have compared these effects across species. Our results showed different T_b and θ_{50} values among three species which potentially lead to large differences on germination timing. According to data-loggers, snow cover was thick enough to constrain freezing temperatures at soil surface level the beginning of January. At this time, seeds of *Festuca halleri* started to accumulate heat ($T_b > T_{env}$) and thermal heat threshold was reached soon, thus germination was estimated by March 3rd (P3). By these dates, snow cover was thicker, consequently the lowest temperature registered was -2.2°C, which is above the frost mean temperature for alpine seedlings to survive (i.e. -2.5°C, Marcante et al. 2012). The ability to germinate under snow has been observed in other high-mountain species (Shimono & Kudo 2005; Fernández-Pascual et al. 2017). This early germination strategy will be an advantageous trait in habitats with short growing season allowing seedlings to grow and establish during the warmer season and to reach an optimum size for overcoming autumn and winter. Nevertheless, germination requirements of *Achillea moschata* were met latter, at the beginning of May (i.e. 11th), as soon as snow cover melted, water was likely available and temperatures started increasing, 254 days after

Table 1. Estimated T_b for each percentile of the population and mean population T_b after the different cold stratification treatments.

Species	Stratification Time(d)	T_b (°C) for proportions of the seed population										T_b (°C)		
		20%	R ²	35%	R ²	50%	R ²	65%	R ²	80%	R ²	Mean	SD	p.value
<i>Achillea moschata</i>	0	12.71*	0.61	12.01*	0.82	11.86*	0.85	11.67*	0.87	10.83*	0.95	11.82	0.30	4.383e- 14
	30	5.56	0.96	5.26	0.99	5.16	0.99	2.57	0.99	5.210	0.99	5.10	0.58	
	60	2.47	0.87	3.26	0.89	3.88	0.91	4.61	0.93	4.67	0.98	3.78	0.42	
	90	3.52	0.89	2.51	0.86	1.47	0.79	0.42	0.72	2.10	0.64	2.00	0.52	
	150	-2.37	0.86	0.15	0.89	1.75	0.92	1.82	0.94	2.36	0.97	0.62	0.83	
<i>Festuca halleri</i>	0	4.77*	0.98	4.57*	0.96	4.51*	0.94	4.42*	0.92	4.69*	0.97	4.59	0.06	1.3e-08 ***
	30	2.98*	0.75	2.50*	0.81	2.33*	0.84	2.17*	0.86	-0.53*	0.72	1.89	0.62	
	60	-1.68	0.99	0.18	0.98	1.02	0.93	1.03	0.89	-	-	0.14	0.53	
	90	1.15	0.99	1.24	0.78	0.60	0.71	-0.01	0.7	0.62	0.72	0.72	0.23	
	150	-1.88	0.85	-1.73	0.86	-1.66	0.87	-1.61	0.87	-1.45	0.89	-1.68	0.80	
<i>Pulsatilla vernalis</i>	0	10.48*	0.11	10.19*	0.04	10.08*	0.018	9.89*	0.02	9.64*	0.23	10.06	0.14	0.144
	30	3.62*	-0.19	7.90*	0.31	8.25*	0.46	8.77*	0.69	9.41*	0.922	7.59	1.02	
	60	8.43*	0.97	7.18*	0.99	6.63*	0.98	9.06*	0.811	8.54*	0.59	7.97	0.46	
	90	6.24*	0.93	9.26*	0.87	9.29*	0.88	9.38*	0.91	9.58*	0.95	8.75	0.63	
	150	5.40*	0.96	9.06*	0.81	9.02*	0.79	9.01*	0.79	9.99*	-	8.5	0.80	

*Species T_b was estimated with the low percentiles (t10,t20,t25,t35 and t50)

Figure 1. Thermal models calculated as the average value of the x-intercepts of the percentiles (average population T_b) after 90 days of cold stratification for the studied species.

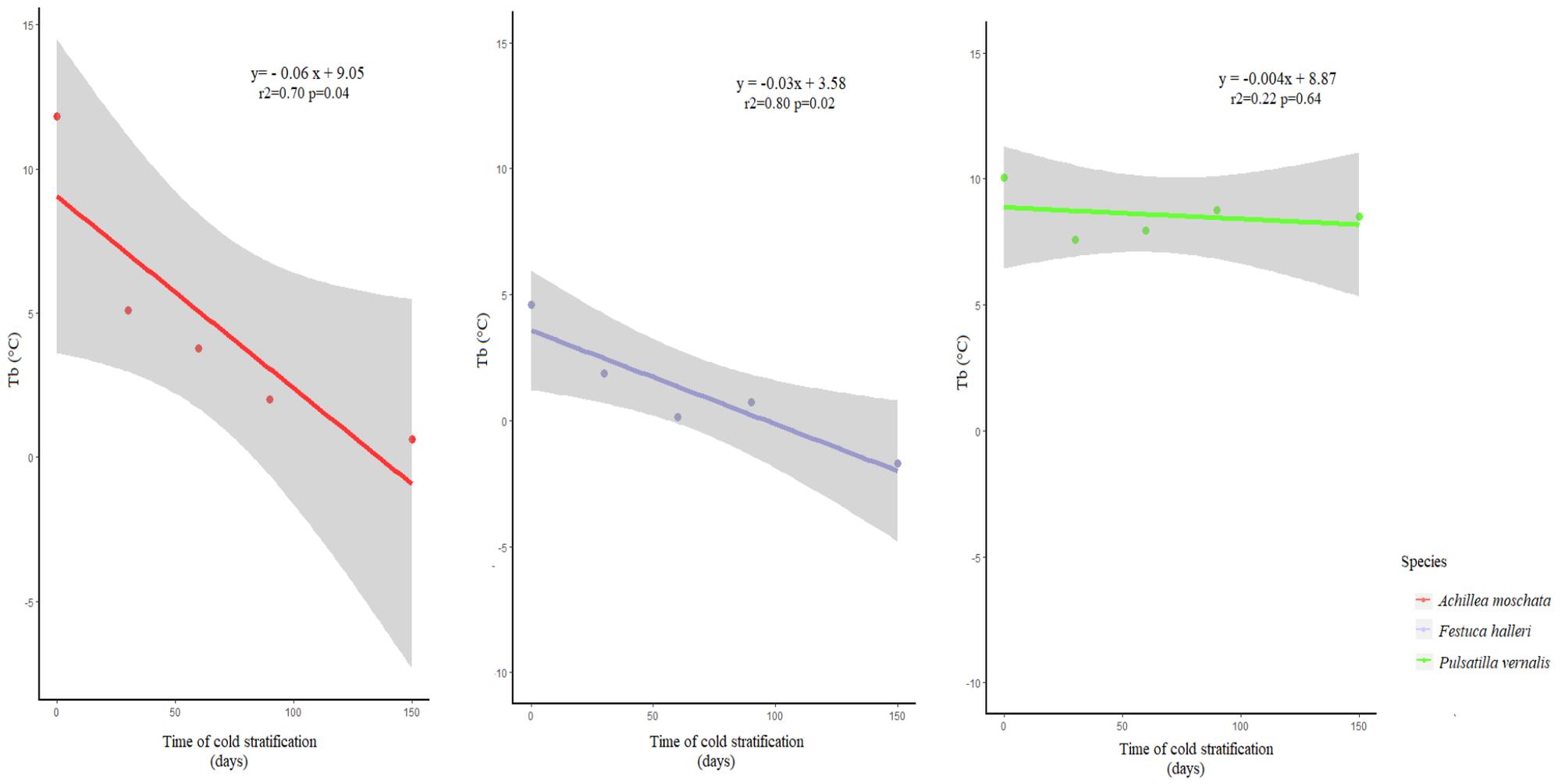
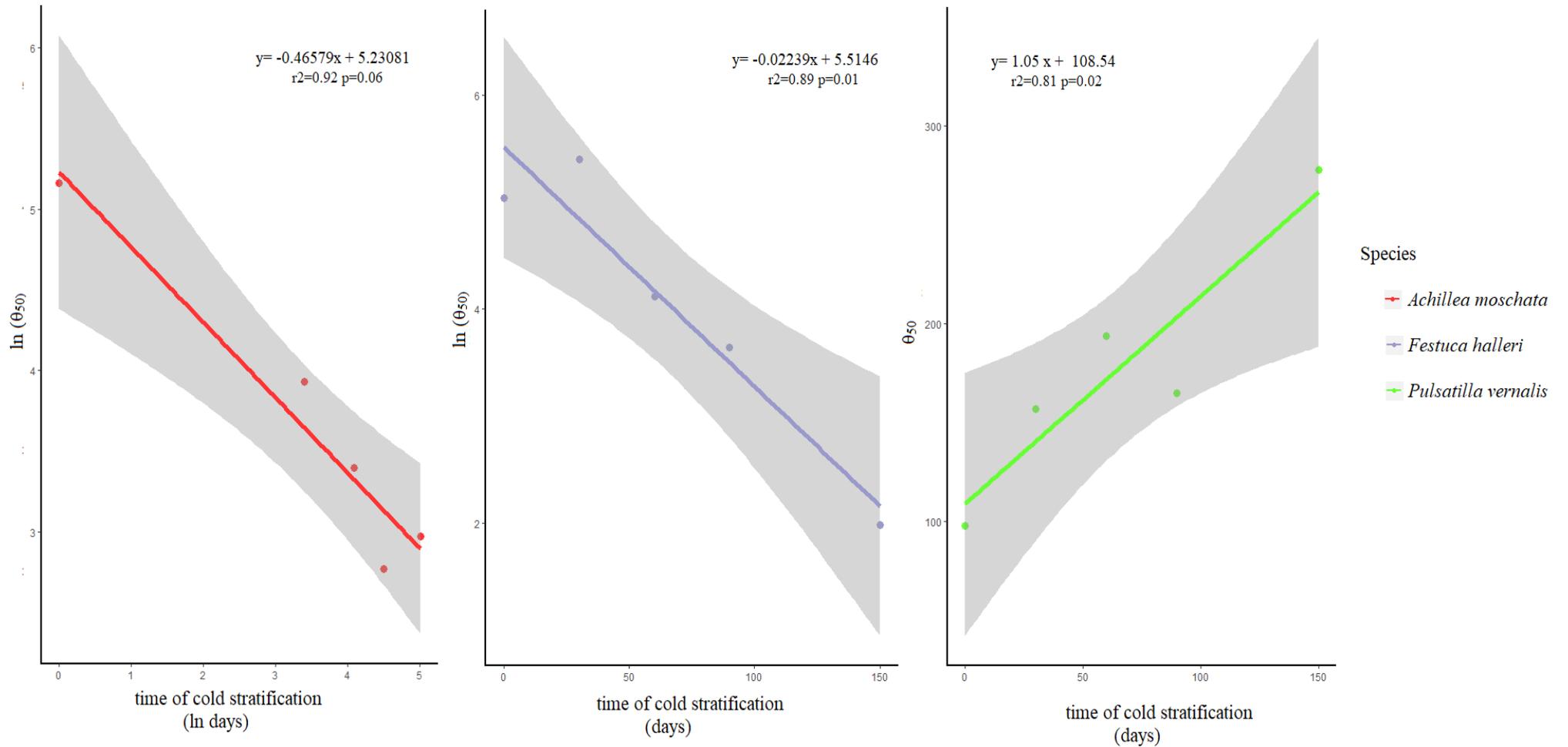


Figure 2. T_b decreasing rate in relation with time of cold stratification.

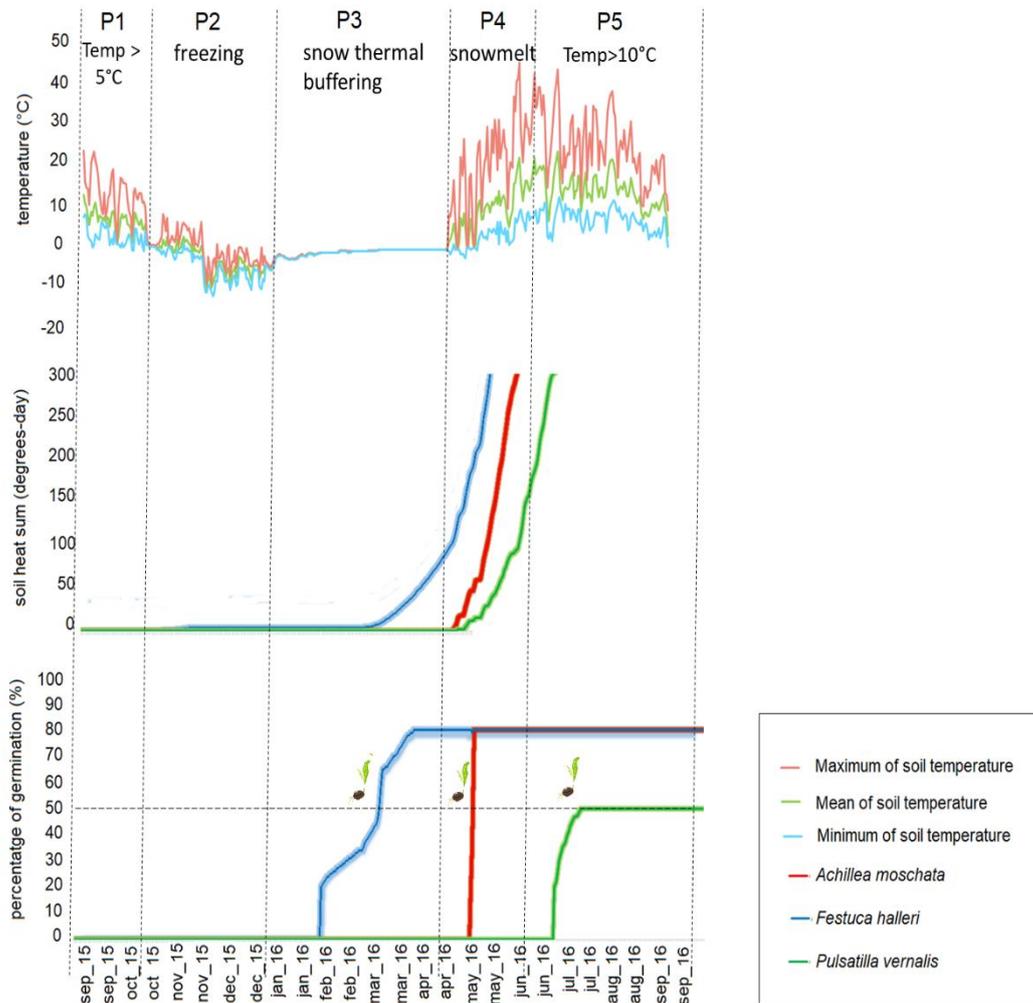


after dispersal (i.e. P4). This relatively faster germination has been considered as the optimum for alpine germination (Körner 2003; Rosbakh and Poschlod 2015), allowing seedlings to escape from low temperatures of winter, while at the same time optimizing the whole growing season. Finally, the germination requirements of *Pulsatilla vernalis* were not reached until day 322 (July 18th) after dispersal (i.e. P5; Figure 3). Although we expected an early germination right after dispersal, the requirements for germinations were not reached until the following summer (almost 10 months after dispersal). Interestingly, germination was postponed until warmer temperatures were guaranteed, which indicate favourable conditions for further seedling recruitment (Fenner & Thompson 2005). Freezing temperatures during the early growing season is the dominant factor causing seedling mortality in not frost resistant alpine flora (Shimono & Kudo 2005), probably including also *Pulsatilla vernalis*.

Our results demonstrate how species that thrive under similar environmental conditions show different germination strategies, suggesting that other factors besides abiotic constraints are affecting seed regeneration. Plant distribution and competitiveness are conditioned by species' niches (Bykova *et al.* 2012) and regeneration niche is an essential component of species' niche (Grubb, 1977). The differentiated germination timing may reduce probability to overlap species' germination niches in alpine environments, hence promoting niche differentiation. Species with different timing do not compete for the same resources (i.e. light, nutrient) during the same month, avoiding competence that might enhance species co-existence in this low productive habitat. Following this view, the late germination timing of *Pulsatilla vernalis* (known as a particularly weaker competitor, Zielińska *et al.* 2016), may allow regeneration niche partitioning, with seedlings not competing with early germinators (such as those of *Festuca halleri*, *Achillea moschata*) which will be already grown up.

Interestingly, *P. vernalis* has a wide biogeographical range and can live in boreal lowland habitats as well (Aeschiman *et al.* 2004), while the other two species are restricted to high altitudes of the European Alps. This suggests that the germination of these species is also linked with their natural history (Schaal and Leverich, 1981) as a result of natural selection in germination traits (Donohue *et al.* 2010). However, we should be aware that intra-specific variation was not evaluated here and also influenced germination thermal thresholds (Porceddu *et al.* 2013), therefore germination timing of *Pulsatilla vernalis* occurring in a roadside of Scandinavia can be different from the

Figure 3. Mean, maximum and minimum soil temperature at the study site. Down soil heat sum and estimated germination time of target species.



germination timing of population occurring at the European Alps. Germination also may differ within populations in some alpine species (Hoyle et al. 2015), spreading probability of seedling recruitment within population. Although intraspecific variation of germination is still unknown in alpine species, it is likely to be lower than the 30% of variation predicted for within-species variation in plant traits (Funk et al. 2016).

According with previous observations (i.e. Tudela-Isanta et al. 2017), our results showed that freshly harvested seeds from alpine grasslands are dormant or conditionally dormant when dispersal. Dormancy is an innate automatism to synchronize germination at the suitable time of the year when environmental conditions are good to ensure seedling recruitment (Mattana et al. 2011; Carta et al. 2014). The species tested here showed different dormancy types (*sensu* Baskin and Baskin 2014), consequently

response to cold stratification differed. *A. moschata* and *F. halleri* showed non-deep physiological dormancy type II pattern of temperature requirement for germination, hence base temperature for germination decrease with time of cold stratification (Figure 2). This response is expected in species mainly occurring at the alpine belt (Aeschiman et al. 2004), where winter is harsh and long chilling requirements are required for germination (Walck et al. 2011). Meanwhile, *P. vernalis* temperature requirements for germination did not change with cold stratification and T_b remained constant. However, seeds of *P. vernalis* have underdeveloped embryo at dispersal (Martin, 1946; unpublished results), hence they showed morphological dormancy. Embryos of morphologically dormant seeds start to growth once seeds have been dispersed from the mother plants and they normally required moist and warm temperatures (Baskin and Baskin, 2014). Indeed, germination of *Pulsatilla vernalis* was highest at 15°C and 20°C when temperatures were warm enough for embryo growth. Interestingly, thermal time of *P. vernalis* significantly increased with cold stratification time, which suggests that seeds potentially developed physiological dormancy during winter. Since *P. vernalis* is the species with the widest distribution range studied here, harsh winter may not be the only limiting factor threatening the recruitment of this species. Despite the differences detected here, our results support that dormancy play a significant role in controlling *in situ* germination timing, delaying, postponing, or slowing the rate of germination (Hoyle et al. 2015), though always awarding risk-averse strategies to escape winter. Indeed, autumn was not a suitable season for seedling recruitment, when temperatures were often below -10°C (Figure 3). Despite alpine seedling are frost resistance, mean frost resistance temperatures are well above -10°C (Marcante et al. 2012). Consequently, if germination occurs in autumn seedlings will die for frost damage, hence germination is postponed after winter.

Overall, this study supports that seedling recruitment of alpine plants is under strong environmental control, being largely affected by low temperatures, short growing season and soil drought during summer (Billings & Mooney 1968; Marcante et al. 2014). In alpine habitat several studies highlighted changes in both timing (Mondoni et al. 2012) and extent of seed germination and seedling recruitment due to climate warming (Graae et al. 2008; Shevtsova et al. 2009; Hoyle et al. 2013; Kim and Donohue 2013; Bernareggi et al. 2016; Fernández-Pascual et al. 2017). However, conclusions are not consistent among studies and yield ambiguous results. In this regard, here we

highlighted that such inconsistencies can be related with the multiple germination strategies in alpine species (including the timing of emergence), thereby supporting an individual response of species' regeneration niche to climate change. For example, seed germination of species with a weaker dormancy might be shifted from summer to autumn in response to increasing temperature (Mondoni et al. 2012), hence thermal units required for germination would be accumulated sooner ($T_b > T_{env}$).

Therefore, further studies including long-term demographic monitoring and *in situ* experiments are needed to clarify how the recruitment of alpine species will be affected by climate change. We also need to understand at what extent co-existing species from alpine plant communities use different timing to reduce competition or optimize the use of resources in the regeneration stage.

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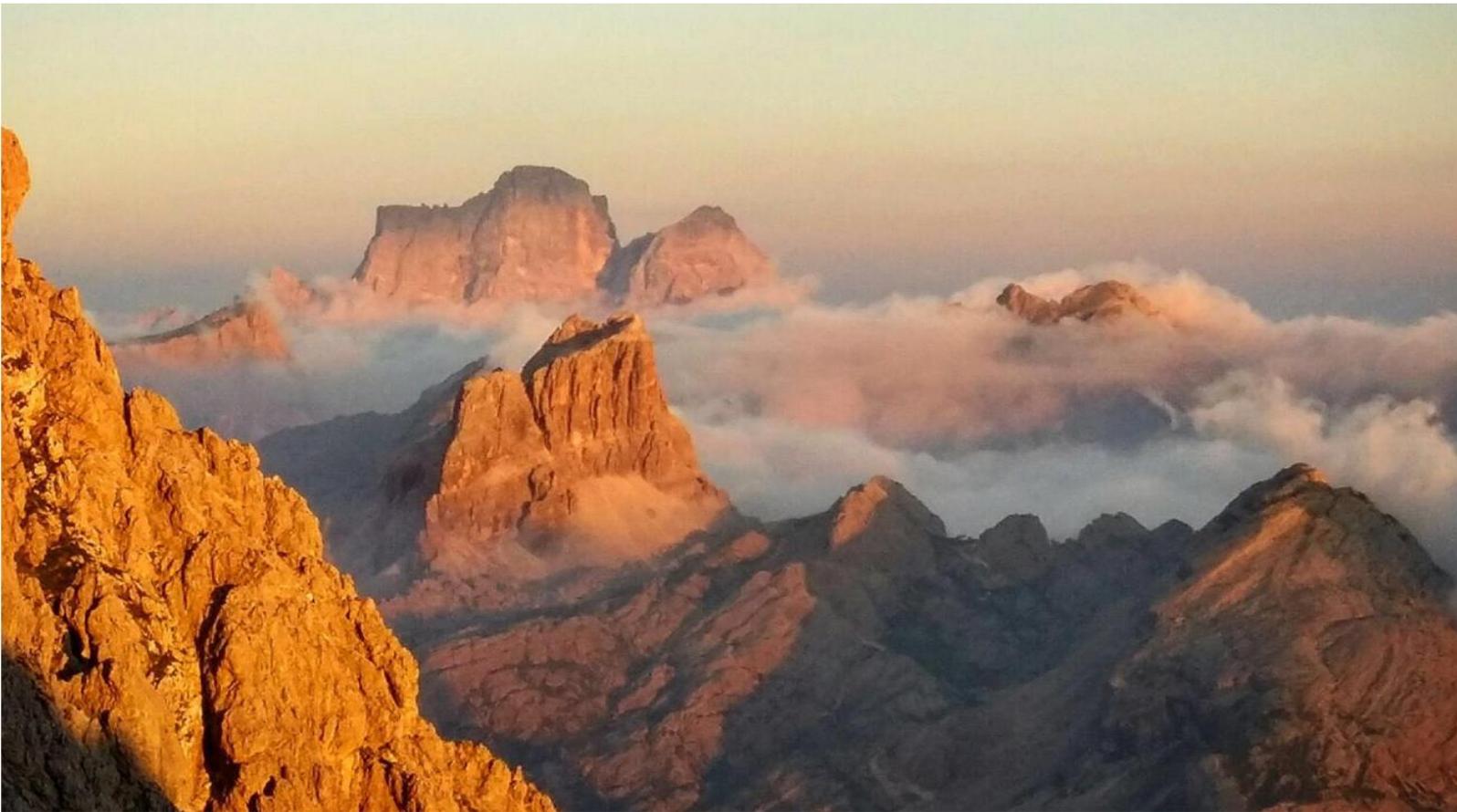
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Chapter 5

General discussion



This thesis focused on the germination ecology of alpine flora and the potential impacts that this plant traits may have on species assembly and distribution. In this study, the dormancy class and type of 53 alpine species was studied. Moreover, the main germination strategies in calcareous and siliceous alpine habitats were assessed and compared. Secondly, vegetative and germination traits of 7 closely related species inhabiting in calcareous and siliceous habitats were defined and compared. Finally, germination timing of three species was estimated using thermal models and soil temperature data.

5.1 Habitat-related germination strategies

Our results showed interspecific differences in germination phenology between and within habitats. However, habitat-related germination strategies have been identified and attributed to the different environmental filters constraining germination in each habitat. Indeed, germination cuing is extremely regulated by the environment (Fenner and Thompson 2005), resulting in the existence of specific germination requirements for many species. In this study, species from calcareous habitat showed deeper dormancy state at dispersal, lower Ψ_b and higher FGP during cold stratification than species from siliceous ones. All these germination traits seem to be adaptive strategies to successfully survive in dry environments. Conversely, species from siliceous environments showed higher germinability (i.e. high FGP) than those from the calcareous once when incubated at acidic pH and lower T_b . As a result, these species germinating under acidic pH and with low T_b have been positively selected to survive and establish in siliceous habitat. The higher capacity of siliceous species to germinate under acidic pH supports the hypothesis that these species are adapted to survive in specific conditions of that habitat (Keddy 1992). Moreover, siliceous plant communities are rarely affected by water limitation (Kammer and Mohl 2002), and consistently not extremely low Ψ_b were identified in species from siliceous bedrocks. In line with that, moister soils are also fresher because of their higher thermal conductivity (Graham *et al.* 2010). Consequently, species from siliceous bedrocks showed lower T_b than the calcareous ones. Indeed, initial temperature of seed germination (T_b) was strongly correlated with habitat temperature (Rosbakh and Poschlod 2015).

Furthermore, different seed dormancy patterns were observed when classifying the species by their main microhabitat. This may suggest that dormancy levels functionally contribute to

species distribution among microhabitats. In this regard, dormancy levels may be associated with environmental heterogeneity (Ooi 2012), with species from more unstable environments showing higher levels of dormancy. For example, seed dormancy was weaker in seeds from *Sorbus aucuparia* inhabiting in higher altitudes than seeds collected from lower altitudes (Barclay and Crawford 1984). Our results demonstrate that changes in the topography at the same elevation have an effect on local environmental stability (Scherrer and Körner 2009), influencing the evolution of different germination strategies amongst species. Therefore, seed dormancy responds to climate variability at a reduced geographical scale, both in the long and short term. Nevertheless, *in situ* experiments are needed to fully understand the role of microclimate (including temperature, water potential, pH and nutrient availability) in driving recruitment success, hence plant community assembly.

5.2 Functional contribution of germination traits.

Functional traits are now extensively used as universal tools in understanding mechanisms behind vegetation patterns from global to local scales (Funk *et al.* 2016). Nevertheless, regenerative traits are not frequently used (Jiménez-Alfaro *et al.* 2016) and germination traits are almost neglected. However, seed germination is crucial step in plant life cycle (Donohue *et al.* 2010; Rosbakh and Poschlod 2015). Moreover, the habitat-related germination strategies/traits and the microhabitat-germination trait relationships identified here may suggest that germination traits are essential in understanding plant distribution patterns at local scale. Indeed, the functional significance of germination traits in community assembly has been already shown (Rosbakh and Poschlod 2015; Huang *et al.* 2016).

For instance, germinability can be interpreted as the species' colonization capacity (Jiménez-Alfaro *et al.* 2016). In our study cases, species with no specific microhabitat (or generalist) had significantly higher Axis I (or germinability) scores than species inhabiting grasslands. The high germination capacity of these species can be interpreted as a bet-hedging strategy, avoiding specialization to optimize their overall fitness in a variable and unpredictable environment (Simons 2011). However, more investigations are needed to verify if the stagger germination reflects differences among, not within, individuals (Starrfelt and Kokko 2012). Meanwhile, the narrower and lower germination conditions showed by most of the species occurring in grasslands may indicate that low germinability acts to guarantee the long-term survival under strong inter and intra-competition among plants. With higher plant densities, the

probability of two species occupying the same ecological niche increase and species co-existence becomes more challenging. For this reason, grassland species tend to produce dormant seeds. Germination is then staggered during the time, allowing potentially competitive species to coexist (Facelli *et al.* 2005), as their ecological germination niches overlap less. Ungerminated seeds will be incorporated in the soil seed bank (Schwienbacher *et al.* 2010), providing an ecological advantage by avoiding unfavorable environmental conditions for seedling establishment (Ooi 2012).

Another interesting point highlighted here, is the lack of correlation found between seed weight and (micro) habitat or germination traits. Nevertheless, seed weight is the most frequently used plant trait in plant community ecology studies. The low informative value identified here when using seed weight to predict species' habitat or microhabitat, together with the lack of correlation found between germination traits and other vegetative traits (Hoyle *et al.* 2015) indicate that including representative germination traits in community ecology models will add significant contribution when exploring global plant variability. Moreover, our results show that germination traits contribute on separating species in the multi-variate space according to their habitat, meanwhile, vegetative traits did not. This may suggest that germination traits may be limiting species occurrence before the adult stage and can be more informative than adult trait when studying alpine plant communities. This is a novel and interesting observation underlining that germination niche is a key point of the plant regeneration niche (Grubb 1977), setting community trajectories and driving ecosystem function and services (Fraaije *et al.* 2015). Therefore, it is urgently needed more research on regeneration processes *in situ* and in the laboratory to find out standardized measurements and protocols to measure germination.

5.3 Alpine germination phenology.

To date, alpine germination was thought to mostly occur in late spring after snowmelt, either because temperatures are not warm enough to promote germination at time of seed dispersal (i.e. late summer or autumn) or because some species require cold stratification to release physiological and morphophysiological dormancy (Schwienbacher *et al.* 2011). This behaviour was interpreted as an ecological strategy to prevent seedling mortality due to the severe and long alpine winters (Billings and Mooney 1968; Körner 2003). Nevertheless, our results support partly this statement because only 22 out of 53 species showed these behaviors. Meanwhile, 11.32% (6 out of the 53 species) of the species showed non-dormant seeds and for most of these species, like *Poa alpina*, cold stratification significantly decreases FGP.

Consequently, these species have high probability to germinate right after dispersal. Hence, despite the low temperatures in winter some alpine seedlings can overcome winter and survive until the following spring (Mondoni et al. 2015). Indeed, some alpine seedlings are frost resistance (Marcante et al. 2012b).

Moreover, 23 out of 53 species germinated during the cold stratification treatments indicating that germination may occur also during winter, when the thermal buffering of the snow cover (around 0°C) (Körner 2003; Mondoni et al. 2015) protect seedlings from frost. In fact, 6 species (i.e. *Pulsatilla alpina*, *Senecio doronicum*, *Rhinantus glacialis*, *Phyteuma orbiculare*, *Festuca pumila* and *Anthyllis vulneraria*) germinated up to 40% or more during cold stratification at 0°C (full darkness). Despite most of the alpine species require light for germination (Jaganathan et al. 2015), these may not be the case for the studied species. Moreover, light sensitive species like *Brassica nigra*, *Latuca sativa* and *Brassica kaber* germinated under 173 cm of snow (Richardson and Salisbury 1977), indicating that some light penetrates through the snow. Hence light must not be a limiting factor for germination to occur under the snow.

On the other hand, some species (i.e. *Antennaria dioica*, *Anthoxanthum odoratum*, *Arnica montana*, *Festuca halleri* and *Pilosella officinarium*) showed high germination under all conditions tested, thereby suggesting a prompt emergence regardless the season. Conversely, several species (i.e. 19 out of 53 species) did not germinate at any of the conditions tested. As mentioned, this was interpreted as a mechanism to ensure the formation of a persistent soil seed bank (Shimono and Kudo 2005; Mondoni et al. 2012) or because appropriate cues for germination were not meet (Donohue et al. 2010; Baskin and Baskin 2014).

5.4 Implications in a climate change context.

Seedling recruitment of alpine plants is under strong environmental control, being affected largely by low temperatures, short growing season and soil drought during summer (Billings and Mooney 1968; Marcante et al. 2014), indicating that climate change will inevitably affect recruitment success. Indeed, several studies already highlighted changes in both the timing (Mondoni et al. 2012) and the extent of seed germination and seedling recruitment due to climate warming (Graae et al. 2008; Shevtsova et al. 2009; Hoyle et al. 2013; Kim and Donohue 2013; Bernareggi et al. 2016; Fernández-Pascual et al. 2017) in alpine plants. However, the conclusions are not consistent among studies and have yielded ambiguous results. Such inconsistencies can be related with the multiple germination strategies existing in

alpine habitats and the studies are focused in different alpine flora, probably showing different germination strategies.

In this regard, the different germination behaviours observed amongst the 54 species investigated here, suggest that climate warming may act differently across species. For example, seed germination of species with weaker dormancy state (i.e. generalist), those from “Cluster D” might be shifted from spring to autumn in response to increasing temperature and heat waves in this season (Mondoni *et al.* 2012) allowing only a few seedlings to survive the hazard of winter. Such a threat can further be enhanced by the expected reduction of snow cover and snowfall in a warmer alpine climate (Klein *et al.* 2016), altering the thermal protection to seedling and therefore affecting seedling survival of species germinating right after dispersal (i.e. Cluster B) (Marcante *et al.* 2012; Mondoni *et al.* 2015). Meanwhile, species showing deep dormancy status that did not germinate at all in any of the treatment tested (i.e. Cluster A and those inhabiting in grasslands) will be less affected by warming at dispersal; such species may have high soil seed bank investment. Interestingly, seed germination is thought not to change in response to climate warming in species with long-term seed banks (Hoyle *et al.* 2013).

Moreover, with climate change is also expected an increasing frequency of heat waves (Orlowsky and Seneviratne 2012). These episodes will not only affect the germination patterns in alpine flora, changing the timing of germination (Orsenigo *et al.* 2015) but also will probably decrease water content of the near-surface soil (Seneviratne *et al.* 2010), where seeds usually germinate. Because seed germination significantly reduced under low water potentials, probably to prevent seedling death, most of alpine species will reduced their germination under drought conditions (Orsenigo *et al.* 2015; Walder and Erschbamer 2015). Nevertheless, some species showed relative low Ψ_b , mainly the ones inhabiting in calcareous habitats, hence we cannot rule out the possibility that some species will be able to germinate. Despite the ability of some alpine species to germinate under low moisture levels if water stress persists seedling recruitment might be compromise, since heat tolerance of alpine seedlings is low (Marcante *et al.* 2014). Therefore, further studies, including long-term demographic monitoring and *in situ* experiments are needed to clarify how the recruitment of alpine species will be affected by climate warming.

5.5 Implications for conservation and restoration activities.

One of the outputs of our findings indicates that different species' chorotype resulted on divergence on germination traits. Moreover, habitat-related germination strategies have been also identified which may indicate the importance of the growing environment in short and long term on the germination behavior. Finally, heat sum models estimated different germination timings. For these reasons, we encourage the use of native seeds in restoration activities to optimize the success. Moreover, during this research, we sampled several alpine grasslands, all located in Italian Alps. The aim of the field visits was collection of seeds for laboratory experiments and seeds for their *ex situ* conservation in the seed bank of the University of Pavia (Pavia, Italy). The final count collection reached on 63 collections from different species. Moreover, germination experiments will result in protocols to optimize the germination of the target species. These protocols may be useful in the future to check the viability of the stored collections or to regenerate plants for restoration or reintroduction purposes. We also checked germinability of seeds held at dry room conditions (at 15°C and 15%RH) for one year and compared with the germinability of non-stored seeds (sown at time of dispersal or after 3 months of cold stratification). These results highlighted that, FGP of stored seeds from 30% of the studied species (i.e. 9 out of 29) (See Supplementary material 5.1) was lower in comparison with non-stored FGP. Moreover, all seeds from three species (i.e. *Phyteuma orbiculare*, *Campanula barbata* and *Solidago vigourea*) were considered death after one year of collection, showing low seed longevity. Indeed, seeds of alpine plants are considered short lived (Mondoni *et al.* 2011, Wijayasinghe *et al.* *unpublished results*). Therefore, future work is needed to confirm these effects and to develop an adequate conservation protocols.

5.6 Conclusions

1. The vast majority of alpine species (44 out of 53) produce dormant seeds after dispersal and tend to prefer warmer germination temperatures. In most cases, dormancy breaks on cold overwintering.
2. Four different germination strategies have been identified in each habitat, showing different regeneration strategies. Within these strategies three were common between habitats: species from "Cluster A" incorporate seeds on the soil seed bank, species from "Cluster B" germinate right after dispersal, species from "Cluster D" germinate right after the snowmelt. And one was

specific from each habitat: species from “Cluster C” in siliceous habitat stagger germination during the whole year, meanwhile, the ones from the calcareous germinate slowly after the snowmelt.

3. Germination patterns/strategies across alpine species vary subtly with habitat provenance, species’ microhabitat, and chorology. These seed germination and (micro) habitat associations may play a key role in driving species distribution across environments, hence in shaping plant community composition and dynamic.

4. Habitat-related germination traits have been identified. Species from siliceous habitats have a higher capacity to germinate under acidic pH, meanwhile calcareous species showed lower Ψ_b and higher T_b .

5. Common germination traits among habitats like high T_o and T_c or treatment effect on increasing the range of germination temperatures have been detected. The high T_o found in both habitats may be a requirement for controlling seedling emergence, avoiding germination in periods of high frost probability.

6. Germination traits contribute on separating species in the multi-variate space according to their habitat, meanwhile vegetative traits not. The convergence of vegetative traits shown here hints a narrower trait differentiation among adult plant species attributed to the high species variability existing within habitats. Though, the separate habitat clusters when using germination traits suggest the existence of specific-habitat regenerative strategies that may limit species occurrence before the adult stage.

7. The different dormancy types detected here, indicate that in alpine context, dormancy may play a significant role in controlling the *in situ* germination timing, delaying, postponing, or slowing the rate of germination, though always awarding risk-averse strategies to escape winter and increase the likelihood of seedling recruitment.

8. Germination timing in alpine species was predicted to be after winter because autumn was not a suitable season for seedling recruitment, because November and December were the coldest month. Though, differences in T_b and θ_{50} values among species lead to potentially large differences on species germination timing. The differentiated germination timing reduce probability to overlap species’ germination niche having different timings species do not compete for the same resources (i.e. light, nutrient) at the same month probably to avoid competence.

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Supplementary material



Chapter 2.

Supplementary material 2.1. Germination traits and species microhabitat and chorology for the target species.

Species	Species characteristics		Germination traits	
	Microhabitat ^a	Chorology ^a	Axis I	Axis II
<i>Achillea moschata</i> Wulfen	Rocky	Artic-Alpine	3.081	1.039
<i>Achillea nana</i> L.	Rocky	Artic-Alpine	2.260	1.273
<i>Antennaria dioica</i> (L.) Gaertn.	Generalist	Continental	3.259	-1.812
<i>Anthoxanthum odoratum</i> L.	Generalist	Continental	2.603	-1.935
<i>Anthyllis vulneraria</i> L.	Grasslands	Continental	-3.899	-0.071
<i>Arnica montana</i> L.	Generalist	Continental	3.710	-2.445
<i>Aster alpinus</i> L.	Grasslands	Artic-Alpine	2.985	0.676
<i>Bartsia alpina</i> L.	Grasslands	Artic-Alpine	0.967	2.126
<i>Biscutella laevigata</i> L.	Grasslands	Artic-Alpine	-1.545	-0.810
<i>Campanula barbata</i> L.	Generalist	Artic-Alpine	1.712	1.625
<i>Carex curvula</i> All.	Heaths	Artic-Alpine	-4.192	-0.072
<i>Carex firma</i> Host	Rocky	Artic-Alpine	-2.215	0.282
<i>Carex sempervirens</i> Vill.	Grasslands	Artic-Alpine	-2.884	0.405
<i>Dryas octopetala</i> L.	Generalist	Artic-Alpine	3.315	-0.614
<i>Festuca halleri</i> All.	Rocky	Artic-Alpine	1.844	-0.999
<i>Festuca nigricans</i> (Hack.) K.Richt.	Rocky	Artic-Alpine	2.518	0.820
<i>Festuca pumila</i> Chaix.	Rocky	Artic-Alpine	1.241	-0.149
<i>Festuca nigrescens</i> Lam.	Grasslands	Continental	-0.161	-0.639
<i>Gentiana acaulis</i> L.	Grasslands	Artic-Alpine	-3.430	0.267
<i>Gentiana clusii</i> E.P. Perrier & Songeon.	Grasslands	Artic-Alpine	0.241	1.876
<i>Gentiana nivalis</i> L.	Grasslands	Artic-Alpine	-3.489	0.279
<i>Gentiana punctata</i> L.	Grasslands	Artic-Alpine	-3.368	0.320
<i>Gentiana verna</i> L.	Grasslands	Artic-Alpine	-3.691	0.243
<i>Gentianella anisodonta</i> (Borbás) Á. & D.Löve	Grasslands	Artic-Alpine	-4.086	-0.017
<i>Geum montanum</i> L.	Heaths	Artic-Alpine	1.674	1.901
<i>Helictochloa versicolor</i> (Vill.) Romero Zarco	Heaths	Artic-Alpine	-2.565	0.026
<i>Horminum pyrenaicum</i> L.	Grasslands	Artic-Alpine	3.101	0.253
<i>Leontodon hispidus</i> L.	Grasslands	Continental	2.857	1.159

<i>Leontopodium alpinum</i> Cass.	Grasslands	Artic-Alpine	3.152	0.972
<i>Lotus corniculatus</i> L.	Grasslands	Continental	-3.129	-0.478
<i>Luzula spicata</i> (L.) DC.	Rocky	Artic-Alpine	-3.859	0.160
<i>Minuartia verna</i> (L.) Hiern subsp. <i>verna</i>	Grasslands	Artic-Alpine	-3.768	0.016
<i>Nardus stricta</i> L.	Heaths	Continental	-1.217	1.159
<i>Pedicularis tuberosa</i> L.	Grasslands	Artic-Alpine	-4.177	-0.065
<i>Phleum rhaeticum</i> (Humphries) Rauschert	Grasslands	Artic-Alpine	1.310	-2.395
<i>Phyteuma hemisphaericum</i> L.	Heaths	Artic-Alpine	0.601	1.604
<i>Phyteuma orbiculare</i> L.	Grasslands	Artic-Alpine	-1.315	0.932
<i>Pilosella officinarum</i> Vaill.	Generalist	Continental	3.237	-2.828
<i>Poa alpina</i> L.	Generalist	Continental	-1.655	-3.920
<i>Polygala alpina</i> (DC.) Steud.	Grasslands	Artic-Alpine	0.301	-3.229
<i>Potentilla aurea</i> L.	Grasslands	Artic-Alpine	-2.734	-0.455
<i>Primula farinosa</i> L.	Heaths	Artic-Alpine	1.511	1.818
<i>Pulsatilla alpina</i> L.	Heaths	Artic-Alpine	1.749	2.371
<i>Pulsatilla vernalis</i> (L.) Mill.	Grasslands	Artic-Alpine	-0.200	-0.259
<i>Ranunculus montanum</i> Willd.	Grasslands	Artic-Alpine	-3.720	-0.132
<i>Rhinanthus glacialis</i> Personnat	Heaths	Continental	-4.014	0.009
<i>Scorzoneroides helvetica</i> (Mérat) Holub	Heaths	Artic-Alpine	2.607	0.982
<i>Senecio doronicum</i> (L.) L.	Generalist	Artic-Alpine	3.481	0.675
<i>Serratula tinctoria</i> L.	Generalist	Continental	1.653	0.846
<i>Sesleria caerulea</i> (L.) Ard.	Rocky	Continental	0.627	-3.409
<i>Soldanella alpina</i> L.	Grasslands	Artic-Alpine	3.755	-1.635
<i>Solidago virgaurea</i> L.	Generalist	Continental	3.288	0.450
<i>Valeriana montana</i> L.	Rocky	Artic-Alpine	0.669	1.795

^a All based from environmental code and world distribution by Aeschimann et al. 2004

Supplementary material 2.2. Species germination outputs (including the mean FGP of each specie and the T50) for each treatment.

Species	acronym	weight (g)	0°C	15/5°C						25/15°C							
			FGP (%)	0CS		3CS		5CS		0CS		3CS		5CS		GA3	
				FGP (%)	T ₅₀ (days)												
<i>Achillea moschata</i> Wulfen	ac_mo	0.009	0	5.3	NA	100	93.37	100	152.77	86.3	9.58	100	95.10	100	150.99	97.7	3.61
<i>Achillea nana</i> L.	ac_na	0.013	0	3.4	NA	77.8	99.08	84.8	152.96	37.5	46.25	91.7	94.94	94.4	151.07	37.4	NA
<i>Antennaria dioica</i> (L.) Gaertn.	an_di	0.003	0	64.4	14.95	100	95.17	89.5	152.60	81.5	4.20	82.5	94.26	71.7	157.02	85.0	3.69
<i>Anthoxanthum odoratum</i> L.	an_od	0.022	5.5	56.6	19.16	72.6	100.91	79.9	158.03	81.3	3.21	62.7	102.25	60.1	159.72	64.5	15.70
<i>Anthyllis vulneraria</i> L.	an_vu	0.208	41.0	1.6	NA	5.3	NA	8.6	NA	9.0	NA	6.7	NA	3.8	NA	6.8	NA
<i>Arnica montana</i> L.	ar_mo	0.076	36.8	93.0	12.35	100	93.58	68.0	151.19	90.0	5.79	96.5	90.97	90.0	151.05	100	1.06
<i>Aster alpinus</i> L.	as_al	0.315	1.0	18.1	NA	95.8	96.71	92.4	154.22	86.1	7.05	92.4	94.22	100	152.84	100	6.82
<i>Bartsia alpina</i> L.	av_al	0.013	2.0	0.0	NA	94.1	NA	90.4	NA	0.0	NA	56.7	NA	50.9	NA	20.6	11.40
<i>Biscutella laevigata</i> L.	ba_al	0.284	0	4.6	NA	5.8	97.35	13.1	160.17	73.7	NA	58.2	96.07	31.6	152.06	73.9	NA
<i>Campanula barbata</i> L.	bi_la	0.003	2.5	12.0	NA	80.0	NA	63.3	NA	40.0	15.69	100	105.82	93.3	NA	98.3	15.54
<i>Carex curvula</i> All.	ca_ba	0.111	0	0.0	NA	0.00	103.72	0.0	169.36	0.0	NA	0.0	91.06	0.0	156.86	0.0	5.60
<i>Carex firma</i> Host	ca_cu	0.032	0	2.2	NA	35.6	NA	49.1	NA	50.0	NA	42.2	NA	49.4	NA	79.1	NA

<i>Carex sempervirens</i> Vill.	ca_fi	0.100	0	1.6	NA	16.6	NA	20.0	NA	1.6	NA	10.0	NA	23.7	NA	23.3	14.27
<i>Dryas octopetala</i> L.	ca_se	0.043	0	7.1	NA	100	NA	100	NA	100	NA	91.7	NA	84.4	NA	100	NA
<i>Festuca halleri</i> All.	dr_oc	0.017	0	10.1	70.25	64.1	91.06	57.4	151.06	62.5	3.58	65.0	93.82	53.7	151.13	69.4	5.72
<i>Festuca nigricans</i> (Hack.) K.Richt.	fe_ha	0.048	6.6	13.6	47.73	83.3	97.00	55.6	163.77	58.6	22.69	91.1	99.37	100	161.59	88.9	8.48
<i>Festuca pumila</i> Chaix.	fe_ni	0.030	70.8	28.7	NA	55.0	100.59	61.1	161.55	69.2	17.44	39.2	NA	100	151.06	62.0	16.65
<i>Festuca nigrescens</i> Lam.	fe_nig	0.041	0.18	22.4	NA	32.8	NA	50.0	156.80	70.2	14.13	48.3	NA	50.0	157.30	63.0	7.66
<i>Gentiana acaulis</i> L.	fe_pu	0.017	0	3.3	NA	26.4	96.58	41.7	151.49	1.7	27.51	10.0	95.12	0.0	156.52	10.0	8.55
<i>Gentiana clusii</i> E.P. Perrier & Songeon.	ge_ac	0.012	0	0.0	NA	90.3	NA	96.4	NA	0.0	NA	39.1	NA	52.4	NA	48.3	12.56
<i>Gentiana nivalis</i> L.	ge_an	0.001	0	0.0	NA	35.4	NA	20.4	NA	0.0	NA	20.5	NA	0.0	NA	0.0	NA
<i>Gentiana punctata</i> L.	ge_cl	0.100	0	0.0	NA	25.7	107.24	13.0	164.22	0.0	NA	32.2	NA	15.1	166.83	48.0	NA
<i>Gentiana verna</i> L.	ge_mo	0.002	0	0.0	NA	0.0	94.53	29.5	151.06	0.0	NA	0.0	94.82	24.5	151.05	84.0	NA
<i>Gentianella anisodonta</i> (Borbás) Á. & D.Löve	ge_ni	0.007	11.6	0.0	NA	1.9	NA	14.0	NA	0.0	NA	1.7	NA	7.2	NA	10.0	NA
<i>Geum montanum</i> L.	ge_pu	0.043	8.3	16.2	NA	97.6	NA	84.9	NA	11.9	NA	83.9	NA	81.5	NA	24.2	NA
<i>Helictochloa versicolor</i> (Vill.) Romero Zarco	ge_ve	0.085	10.0	12.1	NA	26.3	NA	34.3	NA	34.9	NA	45.5	NA	35.5	NA	66.4	8.54
<i>Horminum pyrenaicum</i> L.	ho_py	0.035	0	31.2	NA	100	98.82	80.6	162.47	96.5	4.09	100	91.06	96.3	151.93	94.1	1.93
<i>Leontodon hispidus</i> L.	le_al	0.070	0	7.7	NA	100	96.76	92.7	157.05	61.8	0.67	100	91.06	100	150.97	91.2	6.29
<i>Leontopodium alpinum</i> Cass.	le_hi	0.006	0	3.3	NA	100	95.00	100	151.40	98.3	9.05	100	94.07	100	151.06	100	2.04

<i>Lotus corniculatus</i> L.	lo_co	0.290	0	19.3	NA	21.7	NA	18.4	NA	37.5	NA	28.3	NA	5.0	NA	31.4	NA
<i>Luzula spicata</i> (L.) DC.	lu_sp	0.013	0	0.0	NA	0.0	NA	31.7	NA	0.0	NA	0.0	NA	3.3	NA	0.0	NA
<i>Minuartia verna</i> (L.) Hiern subsp. <i>verna</i>	mi_ve	0.007	7.5	1.8	NA	9.2	NA	11.0	NA	6.8	NA	13.3	NA	3.3	NA	10.0	NA
<i>Nardus stricta</i> L.	na_st	0.019	0	0.0	NA	33.0	NA	40.4	NA	3.9	NA	52.8	110.97	64.2	159.00	30.6	NA
<i>Pedicularis tuberosa</i> L.	pe_tu	0.050	0	0.0	NA	1.7	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA	85.0	17.72
<i>Phleum rhaeticum</i> (Humphries) Rauschert.	ph_al	0.013	18.0	56.4	21.22	64.8	NA	50.8	NA	80.3	6.77	74.0	94.20	80.0	152.25	87.5	6.66
<i>Phyteuma hemisphaericum</i> L.	ph_he	0.013	2.5	14.2	NA	100	96.16	84.6	157.42	8.6	NA	75.9	93.33	40.5	NA	100	3.70
<i>Phyteuma orbiculare</i> L.	ph_or	0.007	52.5	6.8	NA	65.4	105.61	30.6	192.00	1.8	NA	31.2	NA	24.2	NA	100	7.91
<i>Pilosella officinarum</i> Vaill.	pi_of	0.007	20.0	1.0	6.76	90.3	95.57	59.4	156.05	86.7	2.34	100	91.06	55.6	151.59	72.7	1.26
<i>Poa alpina</i> L.	po_al	0.028	7.0	62.9	14.20	18.3	120.46	13.8	192.92	88.9	13.60	21.7	NA	16.9	NA	86.8	7.92
<i>Polygala alpina</i> (DC.) Steud.	po_alp	NA	6.0	74.4	13.74	50.0	NA	30.4	NA	71.1	5.61	36.1	NA	35.7	NA	78.1	4.25
<i>Potentilla aurea</i> L.	po_au	0.015	0	1.7	NA	13.4	NA	24.0	NA	13.5	41.99	30.1	NA	25.0	NA	72.6	19.26
<i>Primula farinosa</i> L.	pr_fa	0.003	0	11.7	NA	75.9	101.85	83.4	161.59	21.2	NA	91.7	94.59	76.7	153.38	98.2	6.31
<i>Pulsatilla alpina</i> L.	pu_al	0.166	40.0	0.0	NA	100	100.69	83.3	151.04	6.7	NA	100	97.29	75.6	155.26	100	13.63
<i>Pulsatilla vernalis</i> (L.) Mill.	pu_ve	0.075	0	0.0	NA	27.3	NA	40.7	NA	85.0	17.63	56.8	111.54	60.2	166.87	33.3	NA
<i>Ranunculus montanum</i> Willd.	ra_mo	0.031	6.6	5.0	NA	8.6	NA	7.9	NA	13.7	NA	15.3	NA	3.3	NA	8.6	NA
<i>Rhinanthus glacialis</i> Personnat	rh_gl	0.124	54.2	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA	0.0	NA

<i>Scorzoneroides helvetica</i> (Mérat) Holub	sc_he	0.080	0	5.3	NA	88.3	94.05	79.9	154.37	69.6	2.72	93.3	91.57	100	151.06	95.0	1.78
<i>Senecio doronicum</i> (L.) L.	se_ca	0.142	90.0	25.1	13.10	100	103.81	100	154.40	92.6	14.13	92.6	NA	100	151.06	89.4	2.83
<i>Serratula tinctoria</i> L.	se_do	0.079	0	0.0	NA	98.1	91.06	92.1	150.97	76.5	9.23	100	99.23	100	150.97	98.1	0.97
<i>Sesleria caerulea</i> (L.) Ard.	se_ti	0.008	8.3	68.6	NA	63.4	94.48	21.4	NA	97.6	6.49	11.1	91.06	11.1	NA	94.4	11.81
<i>Soldanella alpina</i> L.	so_al	0.012	0	57.9	28.29	98.0	103.67	95.5	160.40	100	12.80	96.1	99.46	100	157.39	100	12.19
<i>Solidago virgaurea</i> L.	so_vi	0.033	5.0	26.8	NA	100	93.79	100	150.88	100	4.77	100	95.86	95.0	155.65	93.0	5.68
<i>Valeriana montana</i> L.	va_mo	0.043	16.0	9.0	NA	89.1	98.28	78.9	156.88	3.0	NA	47.1	NA	97.8	150.98	45.3	NA

Supplementary material 2.3a. Dichotomous key to the 5 class and level of dormancy of the 53 target alpine species interfered from germination tests and literature (e.g. seed coat permeability and embryo type, see table Supplementary material 1b).

1. FGP_{ocs} at 25/15 and 15/5°C is $\geq 70\%$**ND**
2. FGP_{ocs} at 25/15 and 15/5°C is $\leq 70\%$**3**
3. Is seed coat permeable?
 - 3.1 Seed coat is not permeable.....**PY**
 - 3.2 Seed coat is permeable.....**4**
4. Is embryo fully developed when dispersal?
 - 4.1 Embryo is undifferentiated or underdeveloped when dispersal..... **5**
 - 4.2 Embryo is fully developed when dispersal **7**
5. Is FGP $\geq 70\%$?
 - 5.1 Yes.....**MD**
 - 5.2 No.....**6**
6. Embryo grows during cold stratification (3 or 5 months)
 - 6.1 Gibberellic acid substituted for cold stratification in promoting germination.....**MPD-intermediate complex**
 - 6.2 Gibberellic acid did not substitute for cold stratification in promoting germination..... **MPD- Deep complex**
 - 6.3 Gibberellic acid or cold stratification does not promote germination.....**unknow-MPD**
7. Long periods of cold stratification increase significantly FGP.
 - 7.1 FGP_{ocs} 25/15°C was high..... **non-deep PD**
 - 7.2 FGP_{ocs} 25/15°C was low and gibberellic acid or short periods (3 months) substituted long period (5CS) of cold stratification in promoting germination.....**intermediate PD**
 - 7.3 FGP_{ocs} 25/15°C was low and gibberellic acid or short periods (3 months) did not substitute long period (5CS) of cold stratification in promoting germination..... **deep PD**
 - 7.4 FGP was low in all conditions tested..... **deep PD**

Supplementary material 2.3b. Seed dormancy class and level based on the data of germination experiments and the reference species with a literature-based description of endosperm characteristics, embryo type and water impermeability of the seed coat (PWI).

Species	Family	Embryo type ^a	Endosperm ^b	PWI ^c	Dormancy	
					Class	Level
<i>Achillea moschata</i> Wulfen	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Achillea nana</i> L.	Asteracea	spatulate fully developed	no endosperm	No	PD	Intermediate
<i>Antennaria dioica</i> (L.) Gaertn.	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Anthoxanthum odoratum</i> L.	Poaceae	lateral	starchy endosperm	No	ND	
<i>Anthyllis vulneraria</i> L.	Fabaceae	bent	fleshy endosperm	Yes	PY	
<i>Arnica montana</i> L.	Asteracea	spatulate fully developed	non starchy endosperm	No	PD	Non deep
<i>Aster alpinus</i> L.	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Bartsia alpina</i> L.	Orobanchaceae	undifferentiated	fleshy endosperm	No	MPD	Deep complex
<i>Biscutella laevigata</i> L.	Brassicaceae	n.a	fleshy endosperma	No	ND	
<i>Campanula barbata</i> L.	Campanulaceae	linear underdeveloped	fleshy endosperm	No	MPD	Intermediate complex
<i>Carex curvula</i> All.	Cyperaceae	capitate	starchy endosperm	No	PD	Deep
<i>Carex firma</i> Host	Cyperaceae	capitate	starchy endosperm	No	PD	Intermediate
<i>Carex sempervirens</i> Vill.	Cyperaceae	capitate	starchy endosperm	No	PD	Deep
<i>Dryas octopetala</i> L.	Rosaceae	spatulate fully developed	fleshy endosperm	No	PD	Non deep
<i>Festuca halleri</i> All.	Poaceae	lateral	starchy endosperm	No	PD	Non deep
<i>Festuca nigricans</i> (Hack.) K.Richt.	Poaceae	lateral	starchy endosperm	No	PD	Non deep
<i>Festuca pumila</i> Chaix.	Poaceae	lateral	starchy endosperm	No	ND	
<i>Festuca nigrescens</i> Lam.	Poaceae	lateral	starchy endosperm	No	ND	
<i>Gentiana acaulis</i> L.	Gentianaceae	linear underdeveloped	fleshy endosperm	No	MPD	Intermediate complex
<i>Gentiana clusii</i> E.P. Perrier & Songeon.	Gentianaceae	linear underdeveloped	fleshy endosperm	No	MPD	Intermediate complex

<i>Gentiana nivalis</i> L.	Gentianaceae	linear underdeveloped	fleshy endosperm	No	MPD	Deep complex
<i>Gentiana punctata</i> L.	Gentianaceae	linear underdeveloped	fleshy endosperm	No	MPD	
<i>Gentiana verna</i> L.	Gentianaceae	linear underdeveloped	fleshy endosperm	No	MPD	Intermediate complex
<i>Gentianella anisodonta</i> (Borbás) Á. & D.Löve	Gentianaceae	linear underdeveloped	fleshy endosperm	No	MPD	
<i>Geum montanum</i> L.	Rosaceae	spatulate fully developed	no endosperm	No	PD	Intermediate
<i>Helictochloa versicolor</i> (Vill.) Romero Zarco	Poaceae	lateral	starchy endosperm	No	PD	Intermediate
<i>Horminum pyrenaicum</i> L.	Lamiaceae	n.a	n.a	No	PD	Non deep
<i>Leontodon hispidus</i> L.	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Leontopodium alpinum</i> Cass.	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Lotus corniculatus</i> L.	Fabaceae	bent	little endosperm	Yes	PY	
<i>Luzula spicata</i> (L.) DC.	Juncaceae	broad	starchy endosperm	No	PD	Deep
<i>Minuartia verna</i> (L.) Hiern subsp. <i>verna</i>	Caryophyllaceae	peripheral	conspicuously ^d	No	PD	Deep
<i>Nardus stricta</i> L.	Poaceae	lateral	fleshy endosperm	No	PD	Deep
<i>Pedicularis tuberosa</i> L.	Orobanchaceae	linear underdeveloped	fleshy endosperm	No	MPD	
<i>Phleum rhaeticum</i> (Humphries) Rauschert	Poaceae	lateral	starchy endosperm	No	ND	
<i>Phyteuma hemisphaericum</i> L.	Campanulaceae	n.a	fleshy endosperm	No	PD	Non deep
<i>Phyteuma orbiculare</i> L.	Campanulaceae	n.a	fleshy endosperm	No	PD	Non deep
<i>Pilosella officinarum</i> Vaill.	Asteracea	spatulate fully developed	no endosperm genus	No	ND	
<i>Poa alpina</i> L.	Poaceae	lateral	starchy endosperm	No	ND	
<i>Polygala alpina</i> (DC.) Steud.	Polygalaceae	spatulate fully developed	fleshy endosperm	No	ND	
<i>Potentilla aurea</i> L.	Rosaceae	spatulate fully developed	non-starchy endosperm	No	PD	Intermediate
<i>Primula farinosa</i> L.	Primulaceae	linear fully developed	hard or firm endosperm	No	PD	Non deep
<i>Pulsatilla alpina</i> L.	Ranunculaceae	rudimentary	fleshy endosperm	No	MPD	Intermediate complex
<i>Pulsatilla vernalis</i> (L.) Mill.	Ranunculaceae	rudimentary	fleshy endosperm	No	MD	

<i>Ranunculus montanum</i> Willd.	Ranunculaceae	linear underdeveloped	fleshy endosperm	No	PD	Deep
<i>Rhinanthus glacialis</i> Personnat	Scrophulariaceae	spatulate underdeveloped	fleshy endosperm	No	PD	Deep
<i>Scorzoneroïdes helvetica</i> (Mérat) Holub	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Senecio doronicum</i> (L.) L.	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Serratula tinctoria</i> L.	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Sesleria caerulea</i> (L.) Ard.	Poaceae	lateral	starchy endosperm	No	ND	
<i>Soldanella alpina</i> L.	Primulaceae	linear fully developed	hard or firm endosperm	No	PD	Non deep
<i>Solidago virgaurea</i> L.	Asteracea	spatulate fully developed	no endosperm	No	PD	Non deep
<i>Valeriana montana</i> L.	Valerianaceae	spatulate fully developed	no endosperm	No	PD	Deep

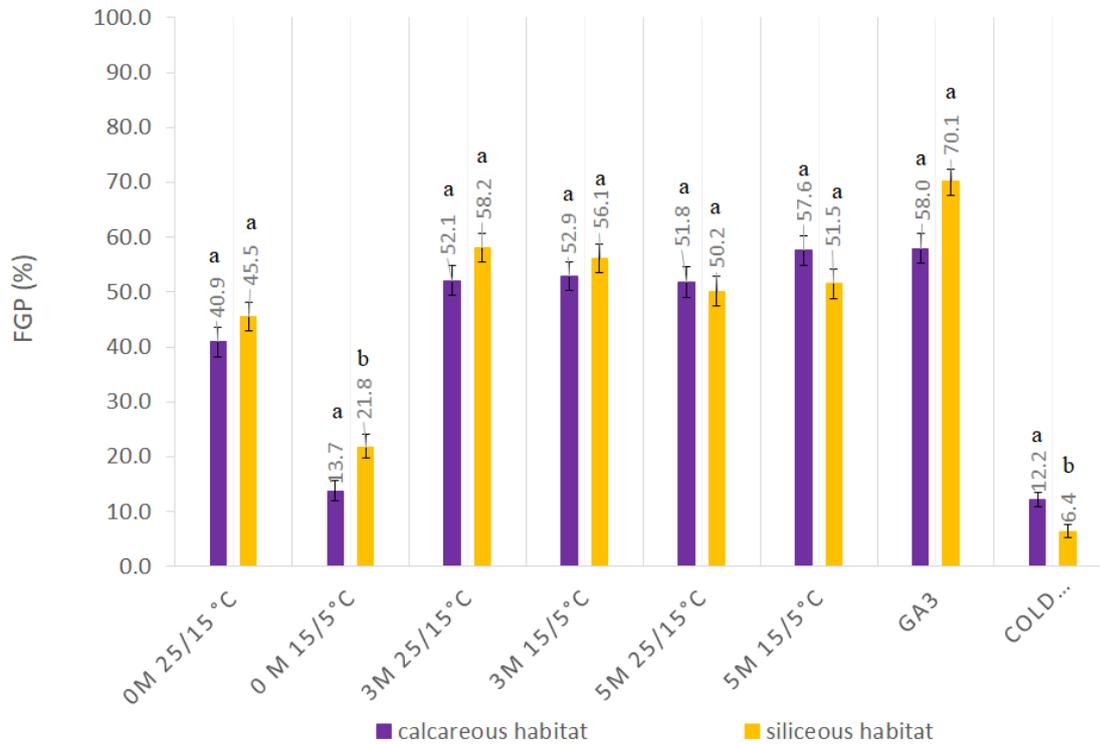
ND= non dormant, PD=physiological dormancy; MD= morphological dormancy;
MPD=morphophysiological dormancy; PY=physical dormancy

^a All from Baskin and Baskin (2007)

^b All from Martin (1946)

^c All from Baskin et al., 2000

Supplementary material 2.4. Final germination percentage (FGP) of both habitats under the two incubation temperatures and treatments **a.** Incubation temperature 15/5° C and 0° C. **b.** Incubation temperature 25/15°C. Significant ($p>0.05$) differences indicate in different letters.



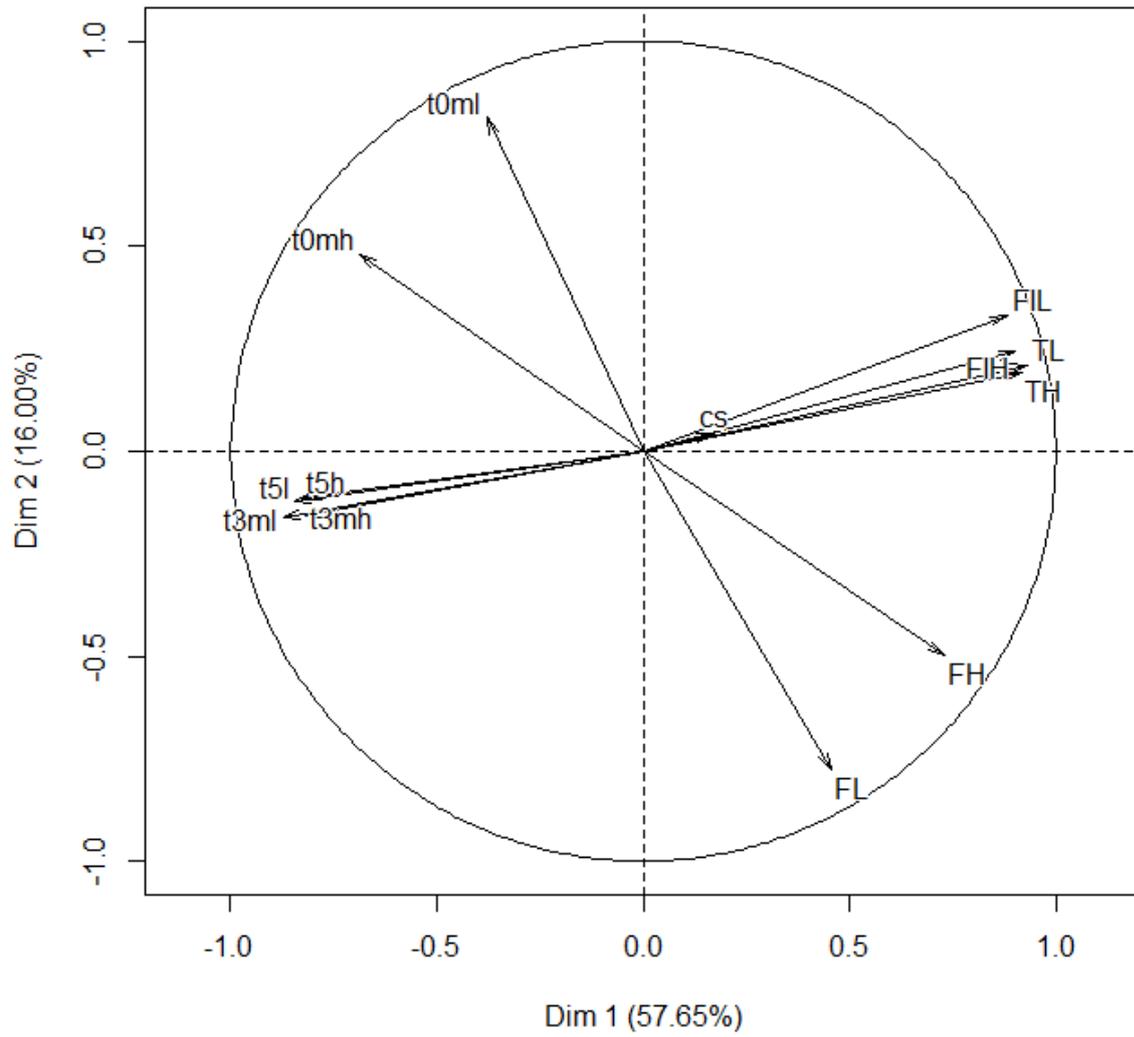
Supplementary material 2.5. Results and variable factor map of the PCA to defined germination traits. The first table represent the eigenvalues and the second the correlation between the variables and the Axis I and II. Finally, there is a graphic representation of the variable factor map from the PCA.

Eigenvalues	Correlation	
	Axis I	Axis II
Variance	7.49	2.07
% of variance	57.65	15.99
Cumulative variance	57.65	73.64

Correlations.

Variables	Correlation	
	Axis I	Axis II
Cold stratification	0.170	0.041
0CS-15/5	0.455	-0.777
3CS-15/5	0.929	0.210
5CS-15/5	0.881	0.332
0CS-25/15	0.728	-0.499
3CS-25/15	0.915	0.190
5CS-25/15	0.900	0.244
T ₅₀ 0CS-25/15	-0.687	0.481
T ₅₀ 0CS-15/5	-0.379	0.813
T ₅₀ 3CS-25/15	-0.818	-0.157
T ₅₀ 3CS-15/5	-0.871	-0.162
T ₅₀ 5CS-25/15	-0.821	-0.112
T ₅₀ 5CS-15/5	-0.846	-0.123

Variables factor map (PCA)



Supplementary material 2.6 a. Results from the ANOVA tests assessing seed weight differences between habitats. Significant ($P < 0.05$) values in bold character. **b.** Results from the lineal models correlating weight and Axis I, Axis II, FGP0M15/5°C and FGP0M25/15°C.

a.

Response variable	λ	AIC	F-value	p.value
Habitat	0.36	97.02	0.017	0.896
Microhabitat	0.39	98.83	0.096	0.908

b.

Response variable	Intercept	Slope	R squared	p.value
Axis I	-0.443	-0.283	-0.016	0.67
Axis II	0.115	0.078	-0.018	0.83
FGP 0M 15/5°C	0.148	-0.017	-0.018	0.79
FGP 0M 25/15°C	0.643	0.061	-0.015	0.63

Chapter 3.

Supplementary material 3.1. Vegetative traits data accessibility for each species resulting from querying TRY- database.

Species	Vegetative traits		
	Source SLA	Source SA	Source LDMC
<i>Achillea moschata</i>	Cerabolini et al. 2010	Cerabolini et al. 2010	Cerabolini et al. 2010
<i>Achillea nana</i>	NA	NA	NA
<i>Campanula barbata</i>	Pierce et al. 2007a; Cerabolini et al. 2010.	Pierce et al. 2007a; Cerabolini et al. 2010.	Pierce et al. 2007a; Cerabolini et al. 2010.
<i>Campanula cochleariifolia</i>	NA	NA	NA
<i>Festuca halleri</i>	Pierce et al. 2007ab; Cerabolini et al. 2010.	Pierce et al. 2007a; Cerabolini et al. 2010.	Pierce et al. 2007a; Cerabolini et al. 2010.
<i>Festuca pumila</i>	NA	NA	NA
<i>Gentiana acaulis</i>	Wright et al. 2004	NA	NA
<i>Gentiana clusii</i>	Cerabolini et al. 2010	Cerabolini et al. 2010	Cerabolini et al. 2010
<i>Phyteuma hemysphaericum</i>	Pierce et al. 2007a; Cerabolini et al. 2010.	Cerabolini et al. 2010; Pierce et al. 2007a	Pierce et al. 2007a; Cerabolini et al. 2010.
<i>Phyteuma orbiculare</i>	Lavergne (2003)	Fitter et al. 1994	NA
<i>Pulsatilla vernalis</i>	Pierce et al. 2007a	Pierce et al. 2007a	Pierce et al. 2007a
<i>Pulsatilla alpina</i>	Cerabolini et al. 2010	Cerabolini et al. 2010	Cerabolini et al. 2010
<i>Geum montanum</i>	Pierce et al. 2007a; Cerabolini et al. 2010.	Pierce et al. 2007a; Cerabolini et al. 2010.	Pierce et al. 2007a ;Cerabolini et al. 2010
<i>Dryas octopetala</i>	Cornelissen et al. 2003; Kleyer et al. 2008	Fitter et al. 1994; Kleyer et al. 2008	Cornelissen et al. 2003; Kleyer et al. 2008; Thompson

LA. Leaf area

SLA. Specific leaf area

LDMC. Leaf dry matter content.

Supplementary material 3.2a. Mean estimated T_b , T_o , and T_c for each species resulting from thermal models using T_{50}

Specie	Habitat	GA ₃ -treated seeds					Untreated seeds				
		T_b	T_o	T_c	R^2_{Sbop}	R^2_{Spop}	T_b	T_o	T_c	R^2_{Sbop}	R^2_{Spop}
<i>Achillea moschata</i>	Siliceous	3.27	31.46	41.54	0.82	0.65	13.46	30.45	38.73	0.70	0.43
<i>Achillea nana</i>	Calcareous	10.26	35.56	40.01	0.83	NA	19.83	29.95	35.00	0.99	NA
<i>Campanula barbata</i>	Siliceous	-1.11	25.73	40.79	0.88	0.85	9.15	14.99	27.62	NA	0.99
<i>Campanula cochlearifolia</i>	Calcareous	5.42	20.07	30.08	0.99	0.99	NA	NA	NA	NA	NA
<i>Festuca halleri</i>	Siliceous	2.92	19.85	40.60	0.83	0.92	4.99	14.89	24.90	0.97	0.99
<i>Festuca pumila</i>	Calcareous	1.60	24.71	35.57	0.93	NA	3.34	14.52	41.62	0.50	0.86
<i>Gentiana acaulis</i>	Siliceous	6.63	20.68	36.58	0.99	0.63	NA	NA	NA	NA	NA
<i>Gentiana clusii</i>	Calcareous	8.16	18.99	35.72	0.76	0.87	NA	NA	NA	NA	NA
<i>Phyteuma hemysphaericum</i>	Siliceous	-0.49	20.08	38.87	0.98	0.92	NA	NA	NA	NA	NA
<i>Phyteuma orbiculare</i>	Calcareous	3.46	21.14	43.54	0.94	0.83	NA	NA	NA	NA	NA
<i>Pulsatilla vernalis</i>	Siliceous	8.99	19.59	30.22	0.77	0.77	6.90	24.80	35.64	0.94	0.91
<i>Pulsatilla alpina</i>	Calcareous	4.41	20.33	31.37	0.91	0.64	NA	NA	NA	NA	NA
<i>Geum montanum</i>	Siliceous	NA	NA	NA	NA	NA	10.01	14.71	29.53	NA	0.93
<i>Dryas octopetala</i>	Calcareous	8.59	24.44	40	0.92	0.94	10.82	25.60	36.30	0.89	0.68

R^2_{Sbop} and R^2_{Spop} . Estimated r squared from the regression lines from T_{50} rates against temperature from the suboptimal and supraoptimal respectively.
 T_b , T_o and T_c . Base, optimal and ceiling temperature for germination
GA3 gibberlic acid

Supplementary material 3.2b. Mean estimated T_b , T_o , and T_c for each species resulting from thermal models using T_{60}

Specie	Habitat	GA ₃ -treated seeds					Untreated seeds				
		T_b	T_o	T_c	R^2_{Sbop}	R^2_{Spop}	T_b	T_o	T_c	R^2_{Sbop}	R^2_{Spop}
<i>Achillea moschata</i>	Siliceous	5.44	31.71	41.56	0.78	0.60	13.46	30.45	38.73	0.70	0.43
<i>Achillea nana</i>	Calcareous	10.89	35.83	40.01	0.77	NA	NA	NA	NA	NA	NA
<i>Campanula barbata</i>	Siliceous	1.33	24.16	40.88	0.79	0.82	NA	NA	NA	NA	NA
<i>Campanula cochlearifolia</i>	Calcareous	6.89	21.32	30.08	0.88	0.99	NA	NA	NA	NA	NA
<i>Festuca halleri</i>	Siliceous	1.66	19.37	42.30	0.65	0.78	4.99	14.9	24.90	NA	0.99
<i>Festuca pumila</i>	Calcareous	1.81	24.79	35.64	0.93	NA	NA	NA	NA	NA	NA
<i>Gentiana acaulis</i>	Siliceous	6.69	20.70	35.63	0.99	0.65	NA	NA	NA	NA	NA
<i>Gentiana clusii</i>	Calcareous	8.39	19.03	31.28	0.77	0.66	NA	NA	NA	NA	NA
<i>Phyteuma hemysphaericum</i>	Siliceous	-0.62	18.76	40.46	0.94	0.94	NA	NA	NA	NA	NA
<i>Phyteuma orbiculare</i>	Calcareous	3.19	20.28	41.67	0.87	0.84	NA	NA	NA	NA	NA
<i>Pulsatilla vernalis</i>	Siliceous	8.86	18.89	33.00	0.73	0.78	6.73	24.74	34.98	0.90	0.86
<i>Pulsatilla alpina</i>	Calcareous	5.25	24.86	30.00	0.98	NA	NA	NA	NA	NA	NA
<i>Geum montanum</i>	Siliceous	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
<i>Dryas octopetala</i>	Calcareous	9.76	28.82	40	0.99	0.94	10.79	25.24	36.08	0.83	0.76

R^2_{Sbop} and R^2_{Spop} . Estimated r squared from the regression lines from T_{60} rates against temperature from the suboptimal and supraoptimal respectively.

T_b , T_o and T_c . Base, optimal and ceiling temperature for germination

GA3 gibberlic acid.

Supplementary material 3.3. Mean estimated Ψ_b for each species resulting from hydromodels using T₅₀ and T₆₀.

Specie	Habitat	Cold-treated seeds		Cold-treated seeds	
		T ₅₀		T ₆₀	
		Ψ_b	R ²	Ψ_b	R ²
<i>Achillea moschata</i>	Siliceous	-0.42	0.99	-0.42	0.99
<i>Achillea nana</i>	Calcareous	-0.39	0.96	-0.40	0.99
<i>Campanula barbata</i>	Siliceous	NA	NA	NA	NA
<i>Campanula cochleariifolia</i>	Calcareous	-0.62	0.90	-0.59	0.91
<i>Festuca halleri</i>	Siliceous	-0.54	0.53	-0.46	0.67
<i>Festuca pumila</i>	Calcareous	-0.62	0.97	-0.61	0.96
<i>Gentiana acaulis</i>	Siliceous	-0.49	0.99	-0.48	0.99
<i>Gentiana clusii</i>	Calcareous	-0.76	0.99	-0.76	0.99
<i>Phyteuma hemysphaericum</i>	Siliceous	-0.51	0.99	-0.48	0.98
<i>Phyteuma orbiculare</i>	Calcareous	-0.60	0.91	-0.57	0.87
<i>Pulsatilla vernalis</i>	Siliceous	-0.47	0.99	-0.48	0.99
<i>Pulsatilla alpine</i>	Calcareous	-0.70	0.71	-0.72	0.45
<i>Geum montanum</i>	Siliceous	NA	NA	NA	NA
<i>Dryas octopetala</i>	Calcareous	-0.53	0.48	-0.54	0.62

R². Estimated r squared from the regression lines from T₆₀ rates against water potential.

Ψ_b : base water potential.

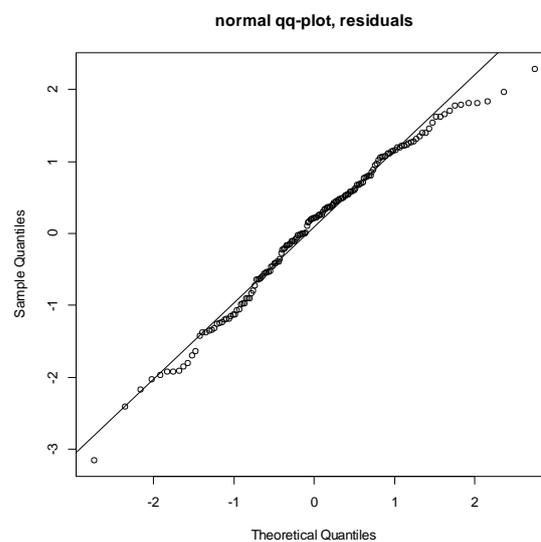
Supplementary material 3.4 Generalized lineal mixed model analysis outputs.

AIC. 763.00

Response variable	Differential	Mean squared	p.value
pH	3	3.86	0.0312
Habitat	1	12.04	0.0034

Scaled residuals

Min	1Q	Median	3Q	max
-3.59	-0.65	0.22	0.75	2.44

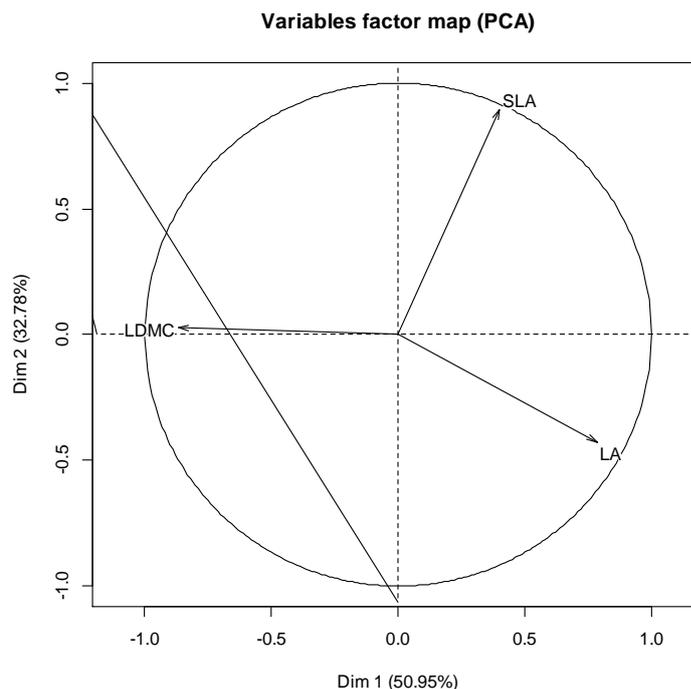


Supplementary material 3.5. Results and variable factor map of the PCA built using vegetative traits. The first table represent the eigenvalues and the second the correlation between the variables and the Axis I and II. Finally, there is a graphic representation of the variable factor map from the PCA.

Eigenvalues	Correlation	
	Axis I	Axis II
Variance	1.52	0.98
% of variance	50.95	32.77
Cumultative variance	50.94	83.72

Correlations.

Variables	Correlation	
	Axis I	Axis II
LDMC	-0.866	0.025
LA	0.785	-0.427
SLA	0.400	0.894



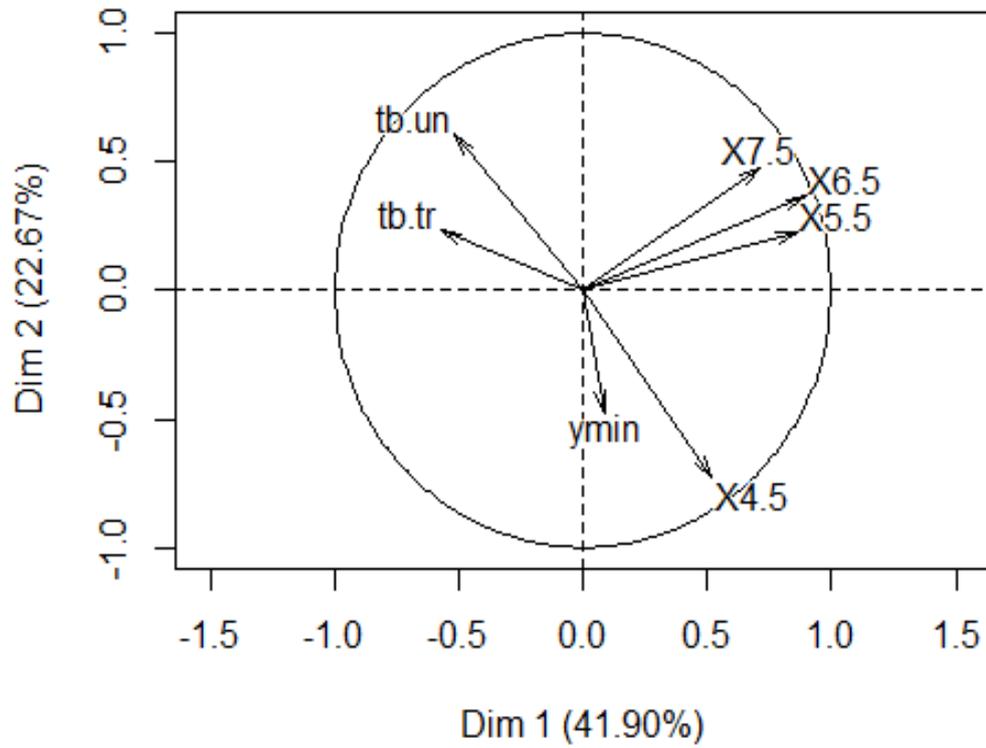
Supplementary material 3.6. Results and variable factor map of the PCA built using germination traits. The first table represent the eigenvalues and the second the correlation between the variables and the Axis I and II. Finally, there is a graphic representation of the variable factor map from the PCA.

Eigenvalues	Correlation	
	Axis I	Axis II
Variance	2.933	1.59
% of variance	41.90	22.66
Cumulative variance	41.90	64.57

Correlations.

Variables	Correlation	
	Axis I	Axis II
FGP 4.5	0.518	-0.725
FGP 5.5	0.863	0.223
FGP 6.5	0.898	0.365
FGP 7.5	0.711	0.479
T _b treated seeds	-0.574	0.231
T _b untreated seeds	-0.517	0.606
Ψ _b	0.083	-0.475

Variables factor map (PCA)



Chapter 5.

Supplementary material 5.1 Final germination percentages of store and fresh seeds, significant differences between fresh and stored seeds were assessed using glmm and indicated with the p.value <0.05. Finally, behaviour was described as observed tendency (increase or decrease) between fresh and stored seeds.

Species	Fresh	Stored	p.value	Behaviour
<i>Achillea moschata Wulfen</i>	0.867	0.647	0.014	Decrease
<i>Antennaria dioica</i>	0.815	0.959	0.02	Decrease
<i>Arnica montana</i>	0.9	1	0.08	No effect
<i>Aster alpina</i>	0.861	1	0.05	Decrease
<i>Biscutella laevigata</i>	0.738	0.3885	0.00045	Decrease
<i>Dryas octopetala</i>	1	1	0.961	No effect
<i>Festuca quadrifolia</i>	0.692	0.855	0.04	Increase
<i>Horminum pyrenaicum</i>	0.965	0.863	0.07	No effect
<i>Leontopodium alpinum</i>	0.982	0.904	0.15	No effect
<i>Leucanthemopsis alpina</i>	0.803	0.795	0.22	No effect
<i>Pilosella officinarum</i>	0.867	0.793	0.445	No effect
<i>Poa alpina</i>	0.889	1	0.98	No effect
<i>Senecio doronicum</i>	0.926	1	0.16	No effect
<i>Serratula tinctoria</i>	0.765	0.556	0.03	Decrease
<i>Soldanella alpina</i>	1	0.98	0.93	No effect
<i>Solidago vigourea</i>	1	death	6.23E-05	Viability was loose
<i>Achillea nana</i>	0.916	0.946	0.446	No effect
<i>Campanula barbata</i>	0.983	death	6.23E-05	Viability was loose
<i>Primula farinosa</i>	0.916	0.887	0.6634	No effect
<i>Pulsatilla alpina</i>	0.982	0.866	0.0146	Decrease
<i>Carex firma</i>	0.35	0.6	0.0281	Increase
<i>Festuca halleri</i>	0.64	1	0.0002529	Increase
<i>Festuca melanopsis</i>	0.832	0.962	0.05	No effect
<i>Gentiana acaulis</i>	0.816	0.735	0.29	No effect
<i>Gentiana clussii</i>	0.903	0.916	0.8728	No effect
<i>Leontodon hispidus</i>	0.982	0.982	0.9	No effect
<i>Phyteuma hemysphaericum</i>	0.98	1	0.998	No effect
<i>Phyteuma orbiculare</i>	0.65	0	6.23E-05	Viability was loose
<i>Valeriana montana</i>	0.891	1	0.004075	Increase

