Università degli Studi di Pavia
Dipartimento di Scienze della Terra e dell’Ambiente

SCUOLA DI ALTA FORMAZIONE DOTTORALE
MACRO-AREA SCIENZE E TECNOLOGIE

DOTTORATO DI RICERCA IN SCIENZE DELLA TERRA E DELL’AMBIENTE

Francesco Porro

Comparative plant diversity and seed germination patterns of alpine species in the context of climate change

Anno Accademico 2018-2019
Ciclo XXXII

Coordinatore	Tutor
Prof. Roberto Sacchi	Dott. Andrea Mondoni
	Co-Tutor
	Prof. Brigitta Erschbamer

Contents

Abstract

Climate is a major determinant of the distribution of ecosystems on earth and influences the latitudinal and altitudinal distribution of both plant species and vegetation. Over the least decades, global warming has been affecting ecosystems worldwide, especially cold regions such as the arctic and alpine biomes. European mountains are considered hotspots of biodiversity, hosting approximately 20% of the continent's native vascular flora, with a high number of endemic species. Yet, these rich and diverse environments are projected to experience one of the highest rates of climate warming, compared to other regions of the world. Climate change has direct impact on plant species, causing species migration and extinction. Consequence, vegetation dynamics in these cold adapted ecosystems are difficult to predict.

In this Thesis, I firstly investigated the variation of species richness, α-diversity, β-diversity and total cover of plant functional types on an alpine long-term monitoring site (belonging to the GLORIA network, https://www.gloria.ac.at), with the aim to promote a better understanding of climate-driven changes of alpine vegetation. I identified significant increment in plant species richness, along with trend of biodiversity loss and signals of biotic homogenization: Cold-adapted and rare species declined while dominant species like nitrophilous graminoids and shrubs increased. The results obtained highlight that long-term vegetation monitoring activities paired with multiple measures of diversity are required to properly assess biodiversity and to obtain useful indications for future conservation activities in alpine environments.

Secondly, I investigated the germination ecology of 75 alpine plants of interest, to evaluate the role of seed germination as a driver for plant species population dynamics in response to climate warming. One of the key processes in determining the species capacity to migrate, establish and persist is, in fact, seed dispersal, followed by seed germination and seedling development. In alpine plants, seed germination is under strong environmental control, suggesting that climate change will inevitably affect recruitment success. In the present study, I selected 75 alpine plant species which populations either increased, decreased or remained stable in five long-term monitoring alpine study sites (belonging to the GLORIA network), along a time period of 15 years. Increasing species germinated at a broader spectrum of environmental conditions than those of the other groups, and were characterized by higher germination, especially at low temperature after cold stratification treatment). These results suggest that germination during early spring, right after snowmelt, may be an advantage in a warmer climate, promoting seedling emergence when water is more available and drought/heat hazards are low.

Finally, I investigated the relationship between seed germination and environmental cues of 28 species of the genus Saxifraga, with the aim to disentangle their germination response from ecological cues and phylogenetic relatedness. Saxifraga seed germination resulted overall promoted by cold stratification and cool temperatures, while being strongly inhibited by darkness. Germination traits in the genus Saxifraga were overall unconstrained by phylogeny, while they were driven by the species ecological niche, as it was possible to observe different germination strategies depending on species requirements for water availability, soil acidity and texture. These results highlight that microenvironmental variables play a major role than phylogeny in determining the germination strategies across species of this genus.

1. Introduction

Climate is a major determinant of the distribution of ecosystems on earth and influences the latitudinal and altitudinal distribution of both plant species and vegetation (Walter 1984, 1985; Ellenberg 1996; Walter and Breckle 1991, 2002). Over the least 20-30 years, global warming has been affecting ecosystems worldwide, especially cold regions such as the arctic and alpine biomes (Rixen et al. 2014). During the recent years, a great number of studies has focused on the impacts of climate change on organisms and biomes, aiming to forecast their future evolution. Several studies have suggested that climate change will have a large impact on ecosystems' biodiversity (Warren et al. 2013; Bellard et al 2014) and will cause changes in the distribution, physiology and phenology of a wide range of taxa (Parmesan 2006). In addition, climate change is expected to exacerbate the impact of alien invasive and ubiquitous species (Walther et al. 2009), leading to high rates of species extinctions (Bellard et al. 2012).

European mountains are considered to be hotspots of biodiversity (Barthlott et al. 1996), hosting approximately 2,500 vascular plant species (or approximately 20% of the continent's native vascular flora), with a high number of endemic species (Grabherr et al. 2000; Nagy and Grabherr 2009; Grabherr et al. 2010; Väre et al. 2003). Yet, these rich and diverse environments are projected to experience one of the highest rates of climate warming, compared to other regions of the world (IPCC 2014). Consequently, developing correct methods to assess the status of biodiversity in relation to the impacts of climate change and understanding the drivers behind plant population dynamics are crucial for the conservation of these natural areas. Alpine environments are particularly suitable to detect and quantify the effects of climate change on biodiversity, since a moderate change in air temperature may significantly affects the altitudinal distribution of plants and vegetation (Körner 2002). Moreover, it is possible to spot across the Alps, habitats relatively undisturbed by direct anthropogenic influence (Grabherr et al. 2000), that allows the study of impacts caused by climatic change without, or at least with minor, masking effects (Pauli et al. 2001a, 2004).

1.1 Climate change scenarios in the alpine environments

The mean averaged combined land and ocean surface temperature show a warming trend of approximately +1°C (with a likely range of 0.8°C to 1.2°C), over the period 1880–2017, (IPCC 2018). According to the climate change predictions of the recent reports (IPCC 2014, 2018), temperatures is estimated to remarkably increase by up to 3.0 °C by 2100. Furthermore, each of the past three decades have been successively warmer than all the previous decades and the first two decades of the 21st century have been the warmest since the beginning of global climate measurements (IPCC 2013; 2014). In fact, the ten warmest years on records occurred in the time lapse between 1998 and 2018, with the average for 2011-2018 being more than 0.90°C above the late 19th century average (Hansen et al. 2017; Osborn and Jones 2019).

In mountain ecosystems, along with temperature, also precipitation, global radiation, relative humidity and length of snow cover periods will be affected by climate change. In addition to this, also frequency and magnitude of heat waves, floods, droughts, and natural hazards are expected to increase (Nogués-Bravo et al. 2007). For example, warm winters or hot summers may become more frequent towards the end of the 21st century; droughts are likely to become more severe in the future and heat waves would become more frequent, more intense and longer lasting (Cubasch et al. 2001; Karl and Trenberth 2003). In alpine and other cold environments of Europe, temperatures have risen at a rate about twice as large as the northern-hemispheric during the last century, with a mean annual temperature increase of about 2 °C (Auer et al. 2007). This increase has been particularly marked since 1980, with annual mean warming rates of about 0.5 °C per decade (European Environmental Agency 2009). Increased temperatures are expected to lead to more severe drought regimes and to great decrease in snow amount and cover duration below about 1500–2000 m of elevation (Gobiet et al. 2014). In addition, many glaciers have lost almost 50% of their mass between 1850 and 2000 (Zemp et al. 2006), permafrost is thawing and winters with a continuous snow cover of 100 days or more have withdrawn into highest altitudes (Breiling and Charamza 1999; Voigt et al. 2010). Therefore, natural hazards related to glaciers and permafrost retreat are expected to become more frequent (Gobiet et al. 2014). Furthermore, the annual cycle of precipitation is expected to change considerably by the end of the 21st century, increasing during winter and decreasing during summer, particularly in the southern regions of Europe (Gobiet et al. 2014). For example, in Mediterranean alpine environments, a decrease in the precipitation has been forecasted to occur mainly during spring (Nogués-Bravo et al. 2008), with a magnitude of the 5% per century (Brunetti et al. 2006). Moreover, summer warming would lead to more frequent extreme climatic events (Giorgi and Lionello 2008), such as heat waves (Schär et al. 2004), high temperature extremes and drought episodes; at the same time, low temperature extremes in winter have been expected to decrease (Kjellström 2004; Beniston et al. 2007; Kjellström et al. 2007; Giorgi and Lionello 2008).

1.2 Biodiversity and alpine plant responses

During the recent decades, particular attention has been given on the impacts of climatic changes on plant species and vegetation, highlighting that central and south European alpine ecosystems will be remarkably affected by both its direct effects (e.g. changes in abiotic factors like temperature and precipitation levels, both highly correlated with flora biodiversity) and indirect effects (e.g. change of permafrost patterns and of disturbance dynamics). However, how do plant species and vegetation respond to such changes? How will be plant diversity affected by such changes?

Temperatures and precipitations along with extreme climatic events (such as heath waves) have a direct impact on plant species. As observed by several authors, their current and forecasted regimes can lead to increased temperature and/or drought stress, causing changes in the physiological and phenological response in plants (Walter and Breckle 2002; Wahid et al. 2007; Orsenigo et al. 2014, Rosbackh et al. 2017). Theurillat and Guisan (2001) described three essential ways in which mountain plants may respond to climatic changes: i) persistence in the modified climate, ii) migration to more suitable climates, and iii) extinction. The persistence of a species in the modified climate could occur for gradual ecological buffering, phenotypic plasticity or genetic adaptation (Theurillat and Guisan 2001) and strong evidence have already been provided about the change in the timing of plant’s life cycles during the year (Root et al. 2005; Rosenzweig et al. 2008; Thackeray et al. 2010; Anderson et al. 2012). However, evidence gleaned from past climate changes tends to indicate that species are more likely to respond by migration rather than by adapting genetically (Huntley 1991).

High mountains environments are characterized by a steep gradual decrease of mean air temperature with increasing altitude (e.g. 0.55 °C per 100 m in the European Alps) and, as result from the compression of thermal life zones, narrow and different vegetation belts are present. For this reason, early studies suggested significant changes of the alpine biodiversity, caused by an upward migration of species associated with climate warming (Grabherr et al. 1994; Root et al. 2003; Walther et al. 2005). Accordingly, subsequent studies based on the re-visitation of historical sites provided evidences of the predicted upward shift of plant species and a concurrent increase of species numbers at higher altitudes (Grabherr et al. 1995; Klanderud and Birks 2003; Walther et al. 2005; Lenoir et al. 2008; Parolo and Rossi 2008). Nevertheless, the shift of a whole vegetation belt is hardly likely to happen in a short time, because every species in a community tents to react in a different way and with different timing to the climate change, depending on their ecological requirements (Rossi and Parolo 2005; Erschbamer 2007).

Species richness increments were observed by several authors in alpine areas in recent years (Pauli et al. 2012; Rixen and Wipf 2017; Steinbauer et al., 2018; Rogora et al. 2018; Lamprecht et al. 2018). In particular, Pauli et al. (2012) observed changes in vascular plant species richness across all the Europe’s major mountain regions on 66 study sites. Across all 66 summits, the average numbers of species per summit increased from 34.9 to 37.7 in only seven years (2001-2008). It is interesting to notice that both species gains (in the boreal-temperate regions) and losses (in the Mediterranean regions) detected in this study were more pronounced on the lower summits. This suggests that upward shifts of plants are mostly driven by leading edge expansions, with the higher number of new arrivals on the lower peaks, probably resulting from larger nearby pools of potential invaders (Pauli et al. 2012). Indeed, rising temperatures should cause an amelioration of the environmental conditions (e.g. longer vegetative season length, increased nitrogen supply in the soil and reduction of some other ecological constraints), opening ”new ground” for invaders from lower elevations and creating pressure for upward migration of alpine species (Heer and Körner 2002; Schmid 2004). This process supposedly results in the aforementioned increase of species number at high altitudes, but also in local extinctions of those plants that already live near the upper margins of elevation (Theurillat and Guisan 2001; Colwell et al. 2008). In fact, it is generally believed that the risk of extinction under climate change strongly corresponds to the inability of species to shift with suitable habitat (Engler et al. 2009; Ozinga et al. 2009). For instance, isolated arctic relict species living only in a restricted range of habitats, or alpine endemics restricted to tops of low mountains (i.e., those lacking nival belts, mainly in the eastern and lower external Alps, or in the Apennines), which cannot migrate upward, are likely to be severely endangered by extinction (Theurillat and Guisan 2001). Furthermore, the risk of species extinction might be increased also by additional drivers (Casazza et al. 2014), such as the competition with new incoming species, the loss of appropriate environmental conditions for germination and seedling survival and the disparity between the velocity of climate change and the dispersal ability, especially for range-restricted species (Parmesan 2006) that very often are habitat-specialists and weak-dispersers (Casazza et al. 2014). For these reasons, the extinction risk is not evenly distributed among species and endemic species are disproportionately affected by this threat (Dirnböck et al. 2011). On the other hand, species which benefit from climate warming by expanding their ranges upward, are expected to be ubiquitous and/or common in the alpine region (see Jurasinski and Kreyling 2007). Gottfried et al. (2012) demonstrate that in the alpine environments the more cold-adapted species are declining, while the more warm-adapted species are increasing. They also show that this trend may not be apparent at the scale of individual mountains, though at the larger continental scale, a significantly higher abundance of thermophilic species has been observed after seven years of monitoring (2001-2008). The process of species substitution described here is known as thermophilization: a general progressive homogenization of the summits' vegetation resulting in a decrease in biodiversity. Biotic homogenization, defined as the process by which the genetic, taxonomic or functional similarities of regional biotas increase over time, is a multifaceted process that encompasses species invasions, extinctions and environmental alterations (Olden and Rooney 2006). This process can lead to a reduction of spatial biotic diversity (McKinney 2005), despite of the increase of species number present in the area. The homogenization process and the consequent loss of spatial biodiversity has been already recorded in several studies (McKinney and Lockwood 1999; Jurasinski and Kreyling 2007; Britton et al. 2009; Ross et al. 2012). Homogenization should be considered a major threat, especially in those areas presenting high rate of biodiversity, such as the Arctics, the Alps and the Mediterranean alpine and mountain areas that are particularly rich in endemic species (Klanderud and Totland 2005; Casazza et al. 2014).

As a final remark, it is important to underline that climate change it is not the only driver of alpine vegetation changes. in addition to temperatures, precipitation and extreme climatic events, further factors such as eutrophication and grazing, should also be taken into consideration (Rixen and Wipf 2017). Soil nitrogen concentration in natural environmental is related to temperature (Rustad et al. 2001), and it is constantly increasing in alpine and subalpine environments (Rogora et al. 2006), even if at a lesser degree if compared to lowlands (Hiltbrunner et al. 2005). Nevertheless, it has already been recognized as a co-driver for alpine species composition changes, often associated with increments in nitrophilous species abundance (Bobbink et al. 2010). The effect of grazing can be observed up to the highest alpine grasslands. However, it is difficult to define a clear trend in vegetation change as its consequence, even if it could alter alpine vegetation composition profoundly (Rixen and Wipf 2017). In fact, plant species and communities were found to react individually depending on elevation and grazing animals considered (Mayer and Erschbamer 2017).

1.3 Biodiversity Assessment

To assess the biodiversity status of an area, the species richness is considered the simplest value to describe the community and the regional diversity (Magurran 1988; Gotelli and Colwell 2001). However, there are evidences that the use of this value could lead to erroneous conclusions. For example, as reported in Jurasinski and Kreyling (2007), considering only species richness as a biodiversity metric could led to overlook processes responsible for biodiversity loss, such as biotic homogenization, often associated to upward migration of new species increment of species richness.

For this reason, in order to have a deeper understanding of the impacts of climate change on alpine plant communities, a more comprehensive measure of biodiversity should be considered. An eligible approach to quantify biodiversity could be the evaluation of the α-diversity and β-diversity along a temporal gradient and including their relations. These two fundamental descriptive variables of ecology and conservation biology have been respectively defined by Whittaker (1960) as the "average diversity within sampling units" (α-diversity) and ''the extent of change in community composition, or degree of community differentiation, in relation to a complex-gradient of environment, or a pattern of environments'' (β-diversity). α-diversity, calculated starting from the data of a single assemblage, such as a quadrant or a plot, could be measured either by the number of species present at the site (species richness), or by some other functions, such as indices that takes into account the abundance or relative frequencies of the species. One of the most widely used of such indices is the Shannon index of diversity (H) (Shannon and Weaver 1948) (or Shannon-Weaver, or Shannon-Wiener; Legendre et al. 2005). Shannon index is a measure of the amount of uncertainty (entropy), and it is most often calculated as:

$$H = - \sum_{i=1}^{S} (p_i) \log_2 (p_i)$$

In the formula 'S' is the number of species, and p_i is the proportion of characters belonging to the i_{th} species, and it is calculated as:

$$p_i = n_i / N$$

Where n_i is the number of individuals belonging to the i_{th} species, and 'N' the total number of individuals of the assemblages studied. Shannon index quantifies the uncertainty (entropy) in predicting the species identity of an individual that is taken at random from the dataset. The value of the Shannon index is a measure of the amount of uncertainty, so that the larger the value of 'H', the greater the uncertainty, and thus diversity of the assemblage, is. Shannon index value increases with the number of species in the assemblage, and when all the species are equally abundant, p_i values equal 1 / S, and the Shannon index takes the value "log(S)". The more unequal the abundances of the species are, the smaller the corresponding Shannon index value is. If practically all abundance is concentrated to one species, and the other are very rare (even if there are many of them), Shannon entropy approaches zero. Shannon index is only one of many diversity indexes with different properties and sensitivity to species abundances and evenness that have been used in ecology as a measure of diversity. However, methods integrating the sensitivity of diversity estimates to species abundances, such as the diversity profiles based on Hill numbers (Hill,1973), remain currently underused in plant ecological research. Hill numbers are a parametric family of diversity indices and have several advantages over other single diversity metrics (Chao et al. 2014). They are expressed by a clear unit of measure (effective number of species) and can depict simultaneously the values of several diversity indices with different sensitivity to species richness and evenness. As such, they could provide new insight for comparing and interpreting vegetation diversity. Moreover, β-diversity can detect changes not described by species richness (Balvanera 2002; Legendre et al. 2005) and can widen the knowledge on ecosystem processes. Since Whittaker's proposal, β-diversity has been summarized by literally dozens of measures linked to different ways to interpret β-diversity itself and that can be adequately sorted into gradient and non-gradient approaches (Vellend 2001), as it can be seen in various studies, like Wilson and Shmida (1984), Koleff et al. (2003) and Legendre et al. (2005). Many different methods to quantify and express β-diversity had been proposed and used through the years: methods based on the use of several diversity indices like Shannon's entropy, similarity index such as Jaccard (Jaccard 1912) or Sørensen index, and other numerous available measures of compositional dissimilarity. As a result, there have been a lot of confusion about what β-diversity actually is, and researchers are still having a hard time agreeing on which quantitative interpretation of β-diversity is the best one (Tuomisto 2010a). Several recent papers like Jost (2006, 2007), Jurasinski et al. (2009), Moreno and Rodrìguez (2010), Tuomisto (2010a, 2010b, 2010c) and Gorelick (2011), starting from Whittaker's diversity concept, tried to discuss the ambiguities of the terminology and to propose a new, more consistent terminology based on different approaches to diversity analysis (Jurasinski and Koch 2011).

1.4 Data collection and analysis: the long-term monitoring approach

In order to detect the effect of climate change on the biodiversity of alpine vegetation along a temporal gradient, the following two approaches can be chosen to obtain suitable data for the analysis: i) the so called "re-visitation approach", which compare historical and present floristic data coming from bibliography and fieldwork, and ii) the diachronic approach, based on long-term data set collected by sampling permanent plots several times in different periods. Interest on climate change effects on alpine ecosystems is constantly increasing, but empirical evidence on recent variation of alpine vegetation in mountain systems is still scarce, and mostly based on resurveys of historical sites (Grabherr et al. 1994, Vittoz et al. 2008, Steinbauer et al. 2018).

The re-visitation approach has been widely used, producing convincing results for northern Alps in Swiss, Austria and Italy (e.g. Hofer 1992: Grabherr et al. 1994; Pauli et al. 2001b; Burga et al. 2004, Gandini 2004, Parolo and Rossi 2008; Wipf et al. 2013. Rixen and Wipf 2017; Steinbauer et al. 2018). This approach can be very useful to detect the progressive incoming of new species (or the loss of species) in the re-surveyed area. However, historical inventories are only usable if an exact localization and also a reliable identification of the species are possible. In addition, reliable old species inventories are rare and missing for most of the high mountain areas (Grabherr et al. 2010), making this approach not always suitable for detecting changes at large scale. Unlike the re-visitation approach, long-term monitoring allows to compare the data collected in different times and regions and to quantify with more precision the biological and ecological response of individuals of each plant species (Rossi and Parolo 2005; Friedmann et al. 2011). However, this fundamental advantage is countered by the need to conceive a very precise method of sampling, data recording and long monitoring periods, in order to obtain useful datasets.

As reported by Grabherr et al. (2001), long-term datasets are needed to correctly estimate variations in e.g. richness, composition, distribution of alpine plant species due to climate change. Alpine regions are especially suited for long-term observational studies of species range shifts related to climate change. In this context, the GLORIA (Global Observation Research Initiative in Alpine Environments - https://gloria.ac.at/) project aims to establish a worldwide observation network to assess climate change impacts on the plant biodiversity of high mountain ecosystems around the world, along an altitudinal gradient, e.g. from treeline ecotone to the nival zone (Pauli et al. 2001a, 2004). For this project a standardised sampling design, called “Multi-Summit approach”, described in the GLORIA field manual (Pauli et al. 2001a, 2004), has been proposed and adopted all over the world, which is essential for the comparability of data belonging to different mountain regions, or from different periods, for spatial-temporal analysis. In 2001, the GLORIA-Europe project started as a pilot study to establish a long-term observation network to collect floristic and climatic data. The most important mountain areas of Europe have been investigated, with 17 Target Regions (TR), each one including a set of 3 or 4 summits at different altitude, for a total of 66 mountain summits, located in 13 different European nations. From this original group the GLORIA project has spread worldwide, and nowadays comprises more than 130 active Target Regions in all the major mountain systems of the planet. The vegetation data analyzed and discussed in Chapter 2, and to a lesser extent in Chapter 3, have been collected in five GLORIA Target Regions, which were setup in 2001 and then managed, by different Universities and research Institutions.

1.5 Regeneration traits in the context of climate change in alpine environments

Recruitment from seeds and regeneration processes are important for determining plant community properties, including community stability and change (Donohue et al. 2010; Burghardt et al. 2014), i.e. for a species to be present in a plant community, first seeds must be dispersed or present in the soil seed bank. In addition, appropriate environmental conditions suitable for germination, plant growth, survival and persistence must be present (Donohue et al. 2010; Poschlod et al. 2013). Since climate has a large influence on regeneration from seeds (Adler and Hille Ris Lambers 2008), understanding the impacts of global warming on seed germination and seedling survival is crucial to predict plant population dynamics. Indeed, temperature and water supply are not only critical drivers for plant's distribution (Woodward and Williams 1987), but also for seed germination and for breaking seed dormancy. Moreover, early life-stages of plants are expected to be more sensitive to climate change than adult stages, and as such, represent a major bottleneck to plant species recruitment (Lloret et al. 2004; Fay and Schultz 2009; Dalgleish et al. 2010). For these reasons, the climatic alterations that influence ecological cues for recruitment, will likely affect population dynamics (Walck et al. 1997; Diemer 2002; Fitch et al. 2007; Walck and Dixon 2009; Baeten et al. 2010).

The strong correlation between climate and plant regeneration from seeds has often resulted in the evolution of species-specific germination requirements (Fenner, Fenner and Thompson 2005), which play a key role in plant distribution and vegetation dynamics (Silvertown and Charlesworth 2001; Neilson et al. 2005; McGill et al. 2006; Pearson 2006). Due to the harsh environmental conditions of the alpine habitats, such as the long and cold winter, the short growing season and the dry/heat episodes in summer (Billings and Mooney 1968; Marcante et al. 2014), seeds of alpine plants have a limited period to germinate and grow (Körner 1999; Forbis 2003; Shimono and Kudo 2005). For this reason, alpine seeds usually profit by a narrow window for germination, synchronized with environmental cues that maximize chances of survival (Baskin and Baskin 2014). Due to large diversity of environmental constrains, different germination strategies have already been described for alpine plants (see Tudela-Isanta et al. 2018a, b, and references therein). Not surprisingly, different germination response to climate warming have also been observed across alpine species (Briceño et al. 2015). For example, climate warming may lead to a shift from mostly spring to autumn emergence in some alpine species (e.g. in those with non-dormant or conditionally dormant seeds; Mondoni et al. 2012), exposing seedlings to seasons that they may not be adapted to experience. On the other hand, early snowmelt in spring may increase seedling establishment due to the extension of the growing season (Mondoni et al. 2015). Hence, depending on the species, the forecasted changes in ecological cues may preclude, delay, or enhance regeneration from seeds (Walck et al. 2011). Beside these species-specific response, the inconsistent germination responses to climate warming have also been related to differences between habitats (Graae et al. 2009), including moisture availability (Bell and Bliss 1980; Welling and Laine 2002), geology, soil qualities, climate (Tudela-Isanta et al. 2018a,b; Körner 2003), or interspecific differences (Lambrecht et al. 2007).

Changes in recruitment success observed so far in alpine species imply that range shifts or changes in abundance are possible in a future warmer climate, though overall success may be dependent on interactions with shifts in other components of the plant community. Despite this little is known about the role of regeneration from seeds as key plant functional traits driving changes of species presence and/or abundance due to climate warming. Understanding dormancy patterns and germination requirements of species showing, for example, a decline, or increment due to climate warming may provide essential insights to predict population response and model trajectory.

1.6 Aims of the Research

The first step of my research was to develop an effective way to assess the biodiversity patterns of alpine plant communities along a temporal gradient, relying on the vegetation data collected by long-term monitoring projects (i.e. GLORIA). In particular, as reported in **Chapter 2**, to achieve this goal I investigated the summit flora of a GLORIA Target Region, examining variations in species richness, α and β-diversity from 2001 to 2015. I propose a methodology based on the usage of several statistical analysis and diversity indexes (e.g. Hill numbers), to achieve a complete and multi-faceted assessment of the biodiversity status of the studied community. Moreover, changes in term of abundance of plant functional types (e.g. forbs, grasses, shrubs) were also investigated.

Secondly, in **Chapter 3**, I studied the relationship between the population dynamics of alpine plant species observed in nature and their germination traits, in the context of climate change. In particular, I selected 75 species (clustered in 5 groups depending on their population dynamics observed in nature), whose seeds were collected in the surroundings of 5 Gloria Target Regions and I tested their germination along a broad range of temperatures, with and without cold stratification, aimed to investigate the presence of physiological dormancy. I hypothesized that species currently showing persistence or an increase under a natural warming trend would 1) have overall higher germination values, 2) show a wider window of suitable temperature for germination and 3) are characterized by less constrained environmental cues to break potential seed dormancy, while 4) that species showing a decline or that disappeared in the study area after the first vegetation survey, would be characterized by more specific germination strategies and/or dormancy patterns.

Finally, in **Chapter 4**, in order to investigate the relationship between seed germination and environmental cues, disentangling the response from climatic drivers and, potentially, interspecific differences (i.e. differences in germination traits determined by phylogenetic distances from the species), I studied and characterized the germination ecology of 28 species within the genus *Saxifraga*. Seeds of the target species (collected all over the European Alps) were tested at different conditions (temperature, light, cold stratification, response to phytohormone Gibberellic Acid GA_3), to investigate their germination traits and their possible relations with species-specific ecological niche where they occur. Moreover, given the high number of *Saxifraga* species tested, this study provides an estimation of the germination ecology in this genus, which was largely unknown.

1.7 References

- Adler, P. B., and Hille Ris Lambers, J. (2008). The influence of climate and species composition on the population dynamics of ten prairie forbs. Ecology, 89(11), 3049-3060.
- Anderson, J. T., Inouye, D. W., McKinney, A. M., Colautti, R. I., and Mitchell-Olds, T. (2012). Phenotypic plasticity and adaptive evolution contribute to advancing flowering phenology in response to climate change. Proceedings of the Royal Society B: Biological Sciences, 279(1743), 3843-3852.
- Auer, I., Böhm, R., Jurkovic, A., Lipa, W., Orlik, A., Potzmann, R., ... and Nieplova, E. (2007). HISTALP—historical instrumental climatological surface time series of the Greater Alpine Region. International Journal of Climatology,27(1), 17-46.
- Baeten, L., De Frenne, P., Verheyen, K., Graae, B. J., and Hermy, M. (2010). Forest herbs in the face of global change: a single-species-multiple-threats approach for Anemone nemorosa. Plant Ecology and Evolution, 143(1), 19-30.
- Balvanera, P., Lott, E., Segura, G., Siebe, C., and Islas, A. (2002). Patterns of β-diversity in a Mexican tropical dry forest. Journal of Vegetation Science, 13(2), 145-158.
- Baskin, C. C., and Baskin, J. M. (2014). Seeds: ecology, biogeography and evolution of dormancy and germination., (2nd edn)(Academic Press: San Diego, CA, USA).
- Barthlott, W., Lauer, W., and Placke, A. (1996). Global distribution of species diversity in vascular plants: Towards a world map of phytodiversity (globale verteilung der artenvielfalt höherer pflanzen: Vorarbeiten zu einer weltkarte der phytodiversität). Erdkunde, 317-327.
- Bell, K. L., and Bliss, L. C. (1980). Plant reproduction in a high arctic environment. Arctic and Alpine Research, 12(1), 1-10.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., and Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. Ecology Letters, 15(4), 365-377.
- Bellard, C., Leclerc, C., Leroy, B., Bakkenes, M., Veloz, S., Thuiller, W., and Courchamp, F. (2014). Vulnerability of biodiversity hotspots to global change. Global Ecology and Biogeography, 23(12), 1376-1386.
- Beniston, M., Stephenson, D. B., Christensen, O. B., Ferro, C. A., Frei, C., Goyette, S., ... and Woth, K. (2007). Future extreme events in European climate: an exploration of regional climate model projections. Climatic Change, 81(1), 71-95.
- Billings, W. D., and Mooney, H. A. (1968). The ecology of arctic and alpine plants. Biological reviews, 43(4), 481-529.

- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., Bustamante, M., Cinderby, S., Davidson, E., Dentener, F., Emmett, B., Erisman, J.W., Fenn, M., Gilliam, F., Nordin, A., Pardo, L., De Vries, W. (2010) Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecol Appl 20:30–59
- Breiling, M., and Charamza, P. (1999). The impact of global warming on winter tourism and skiing: a regionalised model for Austrian snow conditions. Regional Environmental Change, 1(1), 4-14.
- Briceño, V. F., Hoyle, G. L., and Nicotra, A. B. (2015). Seeds at risk: how will a changing alpine climate affect regeneration from seeds in alpine areas?. Alpine Botany, 125(2), 59-68.
- Britton, A. J., Beale, C. M., Towers, W., and Hewison, R. L. (2009). Biodiversity gains and losses: evidence for homogenisation of Scottish alpine vegetation. Biological conservation, 142(8), 1728-1739.
- Brunetti, M., Maugeri, M., Monti, F., and Nanni, T. (2006). Temperature and precipitation variability in Italy in the last two centuries from homogenised instrumental time series. International Journal of Climatology, 26(3), 345-381.
- Burga, C. A., G. R. Walther, and S. Beissner (2004). "Florenwandel in der alpinen Stufe des Berninagebiets-ein Klimasignal." Berichte der Reinhold Tüxen Gesellschaft 16: 57-66.
- Burghardt, L. T., Metcalf, C. J. E., Wilczek, A. M., Schmitt, J., and Donohue, K. (2014). Modeling the influence of genetic and environmental variation on the expression of plant life cycles across landscapes. The American Naturalist, 185(2), 212-227.
- Casazza, G., Giordani, P., Benesperi, R., Foggi, B., Viciani, D., Filigheddu, R., ... and Mariotti, M. G. (2014). Climate change hastens the urgency of conservation for range-restricted plant species in the central-northern Mediterranean region. Biological Conservation, 179, 129-138.
- Chao A., Chiu C. H., Jost L. (2014). Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. Annual Review of Ecology, Evolution, and Systematics 45, 297-324.
- Colwell, R. K., Brehm, G., Cardelús, C. L., Gilman, A. C., and Longino, J. T. (2008). Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science, 322(5899), 258-261.
- Cubasch, U., Meehl, G. A., Boer, G. J., Stouffer, R. J., Dix, M., Noda, A., ... and Yap, K. S. (2001). Projections of future climate change., in: JT Houghton, Y. Ding, DJ Griggs, M. Noguer, PJ Van der Linden, X. Dai, K. Maskell, and CA Johnson (eds.): Climate Change

2001: The Scientific Basis: Contribution of Working Group I to the Third Assessment Report of the Intergovernmental Panel, 526-582.

- Diemer, M. (2002). Population stasis in a high-elevation herbaceous plant under moderate climate warming. Basic and Applied Ecology, 3(1), 77-83.
- Dirnböck, T., Essl, F., and Rabitsch, W. (2011). Disproportional risk for habitat loss of high-altitude endemic species under climate change. Global Change Biology, 17(2), 990-996.
- Donohue, K., Rubio de Casas, R., Burghardt, L., Kovach, K., and Willis, C. G. (2010). Germination, postgermination adaptation, and species ecological ranges. Annual review of ecology, evolution, and systematics, 41, 293-319.
- Dalgleish, H. J., Koons, D. N., and Adler, P. B. (2010). Can life-history traits predict the response of forb populations to changes in climate variability?. Journal of Ecology, 98(1), 209-217.
- European Environmental Agency. (2009). Regional climate change and adaptation: the Alps facing the challenge of changing water resources. 8, 143.
- Ellenberg, H. (1988). Vegetation ecology of central Europe. Cambridge University Press.
- Engler, R., Randin, C. F., Vittoz, P., Czáka, T., Beniston, M., Zimmermann, N. E., and Guisan, A. (2009). Predicting future distributions of mountain plants under climate change: does dispersal capacity matter? Ecography, 32(1), 34-45.
- Erschbamer, B. (2007). Winners and losers of climate change in a central alpine glacier foreland. Arctic, Antarctic, and Alpine Research, 39(2), 237-244.
- Fay, P. A., and Schultz, M. J. (2009). Germination, survival, and growth of grass and forb seedlings: effects of soil moisture variability. Acta Oecologica, 35(5), 679-684.
- Fenner, M. K., Fenner, M., and Thompson, K. (2005). The ecology of seeds. Cambridge University Press.
- Fitch, E. A., Walck, J. L., and Hidayati, S. N. (2007). Agroecosystem management for rare species of Paysonia (Brassicaceae): integrating their seed ecology and life cycle with cropping regimens in a changing climate. American journal of botany, 94(1), 102-110.
- Forbis, T. A. (2003). Seedling demography in an alpine ecosystem. American Journal of Botany, 90(8), 1197-1206.
- Friedmann, B., Pauli, H., Gottfried, M., and Grabherr, G. (2011). Suitability of methods for recording species numbers and cover in alpine long-term vegetation monitoring. Phytocoenologia, 41(2), 143-149.

- Gandini, M., (2004). Studio floristico dell'area del Rifugio Marinelli-Bombardieri (Alpi Retiche, Italia). Primi riscontri dell'effetto dei cambiamenti climatici. Tesi sperimentale di laurea in Scienze Naturali, Università degli Studi di Pavia.
- Graae, B. J., Ejrnæs, R., Marchand, F. L., Milbau, A., Shevtsova, A., Beyens, L., and Nijs, I. (2009). The effect of an early-season short-term heat pulse on plant recruitment in the Arctic. Polar Biology, 32(8), 1117.
- Giorgi, F., and Lionello, P. (2008). Climate change projections for the Mediterranean region. Global and Planetary Change, 63(2), 90-104.
- Gobiet, A., Kotlarski, S., Beniston, M., Heinrich, G., Rajczak, J., and Stoffel, M. (2014). 21st century climate change in the European Alps—a review. Science of the Total Environment, 493, 1138-1151.
- Gorelick, R. (2011) Commentary: Do we have a consistent terminology for species diversity? The fallacy of true diversity. Oecologia 167, 885-888.
- Gotelli, N. J., and Colwell, R. K. (2001). Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecology Letters, 4(4), 379-391.
- Gottfried, M., Pauli, H., Futschik, A., Akhalkatsi, M., Barančok, P., Alonso, J. L. B., ... and Grabherr, G. (2012). Continent-wide response of mountain vegetation to climate change. Nature Climate Change, 2(2), 111-115.
- Grabherr, G., and Gottfried, M. (86). H. Pauli (1994): Climate effects on mountain plants. Nature, 369, 448.
- Grabherr, G., Gottfried, M., Gruber, A., and Pauli, H. (1995). Patterns and current changes in alpine plant diversity. In Arctic and alpine biodiversity: patterns, causes and ecosystem consequences (pp. 167-181). Springer Berlin Heidelberg.
- Grabherr, G., Gottfried, M., and Pauli, H. (2000). GLORIA: a global observation research initiative in alpine environments. Mountain Research and Development, 20(2), 190-191.
- Grabherr, G., Gottfried, M., and Pauli, H. (2001). Long-term monitoring of mountain peaks in the Alps. In Biomonitoring: General and applied aspects on regional and global scales (pp. 153-177). Springer Netherlands.
- Grabherr, G., Gottfried, M., and Pauli, H. (2009). Climate effects on mountain plants. Nature, 369(6480), 448-448.
- Grabherr, G., Gottfried, M., and Pauli, H. (2010). Climate change impacts in alpine environments. Geography Compass, 4(8), 1133-1153.

- Hansen, J., Sato, M., Kharecha, P., Von Schuckmann, K., Beerling, D. J., Cao, J., ... and Shakun, J. (2016). Young people's burden: requirement of negative CO2 emissions. arXiv preprint arXiv:1609.05878.
- Heer, C., and Körner, C. (2002). High elevation pioneer plants are sensitive to mineral nutrient addition. Basic and Applied Ecology, 3(1), 39-47.
- Hill, M. O. (1973). Diversity and evenness: a unifying notation and its consequences. Ecology, 427-432.
- Hiltbrunner, E., Schwikowski, M., and Körner, C. (2005). Inorganic nitrogen storage in alpine snow pack in the Central Alps (Switzerland). Atmospheric Environment, 39(12), 2249-2259.
- Hofer, H. R. (1992). Veränderungen in der Vegetation von 14 Gipfeln des Berninagebietes zwischen 1905 und 1985. Berichte des Geobotanischen Institutes der Eidgenössischen Technischen Hochschule Stiftung Ruebel, 58, 39-54.
- Huntley, B. (1991). How plants respond to climate change: migration rates, individualism and the consequences for plant communities. Annals of Botany,67(supp1), 15-22.
- IPCC (2013). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y. Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA, 1535 pp.
- IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland.
- IPCC (2018). Global Warming of 1.5° C: An IPCC Special Report on the Impacts of Global Warming of 1.5° C Above Pre-industrial Levels and Related Global Greenhouse Gas Emission Pathways, in the Context of Strengthening the Global Response to the Threat of Climate Change, Sustainable Development, and Efforts to Eradicate Poverty. Intergovernmental Panel on Climate Change.
- Jaccard, P. (1912). The distribution of the flora in the alpine zone. 1. New phytologist, 11(2), 37-50.
- Jost, L. (2006). Entropy and diversity. Oikos, 113(2), 363-375.
- Jost, L. (2007). Partitioning diversity into independent alpha and beta components. Ecology, 88(10), 2427-2439.
- Jurasinski, G., and Kreyling, J. (2007). Upward shift of alpine plants increases floristic similarity of mountain summits. Journal of Vegetation Science, 18(5), 711-718.

- Jurasinski, G., Retzer, V., and Beierkuhnlein, C. (2009). Inventory, differentiation, and proportional diversity: a consistent terminology for quantifying species diversity. Oecologia, 159(1), 15-26.
- Jurasinski, G., and Koch, M. (2011). Commentary: do we have a consistent terminology for species diversity? We are on the way. Oecologia, 167(4), 893-902.
- Karl, T. R., and Trenberth, K. E. (2003). Modern global climate change. science,302(5651), 1719-1723.
- Kjellström, E. (2004). Recent and future signatures of climate change in Europe. AMBIO: A Journal of the Human Environment, 33(4), 193-198.
- Kjellström, E., Bärring, L., Jacob, D., Jones, R., Lenderink, G., and Schär, C. (2007). Modelling daily temperature extremes: recent climate and future changes over Europe. Climatic Change, 81(1), 249-265.
- Klanderud, K., and Birks, H. J. B. (2003). Recent increases in species richness and shifts in altitudinal distributions of Norwegian mountain plants. The Holocene, 13(1), 1-6.
- Klanderud, K., and Totland, Ø. (2005). Simulated climate change altered dominance hierarchies and diversity of an alpine biodiversity hotspot. Ecology, 86(8), 2047-2054.
- Koleff, P., Gaston, K. J., and Lennon, J. J. (2003). Measuring beta diversity for presence–absence data. Journal of Animal Ecology, 72(3), 367-382.
- Körner, C. (1993). Response of alpine vegetation to global climate change. Catena supplement, 22, 85-85.
- Körner, C. (1999). Alpine plants: stressed or adapted. Physiological Plant Ecology. Blackwell, Oxford, 297-312.
- Körner, C. (2002). Impact of atmospheric changes on high mountain vegetation. In Mountain environments in changing climates (pp. 149-157). Routledge.
- Körner, C. (2003). Alpine plant life: functional plant ecology of high mountain ecosystems; with 47 tables. Springer Science and Business Media.
- Lambrecht, S. C., Loik, M. E., Inouye, D. W., and Harte, J. (2007). Reproductive and physiological responses to simulated climate warming for four subalpine species. New Phytologist, 173(1), 121-134.
- Lamprecht, A., Semenchuk, P. R., Steinbauer, K., Winkler, M., and Pauli, H. (2018). Climate change leads to accelerated transformation of high-elevation vegetation in the central Alps. New Phytologist, 220(2), 447-459.
- Legendre, P., Borcard, D., and Peres-Neto, P. R. (2005). Analyzing beta diversity: partitioning the spatial variation of community composition data. Ecological Monographs, 75(4), 435-450.

- Lenoir, J., Gégout, J. C., Marquet, P. A., De Ruffray, P., and Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. Science, 320(5884), 1768-1771.
- Lloret, F., Penuelas, J., and Estiarte, M. (2004). Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Global Change Biology, 10(2), 248-258.
- Magurran, A. E. (1988). Ecological diversity and its measurement (Vol. 168). Princeton: Princeton University Press.
- Marcante, S., Sierra-Almeida, A., Spindelböck, J. P., Erschbamer, B., and Neuner, G. (2012). Frost as a limiting factor for recruitment and establishment of early development stages in an alpine glacier foreland?. Journal of Vegetation Science, 23(5), 858-868.
- Marcante, S., Erschbamer, B., Buchner, O., and Neuner, G. (2014). Heat tolerance of early developmental stages of glacier foreland species in the growth chamber and in the field. Plant ecology, 215(7), 747-758.
- Mayer, R., and Erschbamer, B. (2011). Seedling recruitment and seed-/microsite limitation in traditionally grazed plant communities of the alpine zone. Basic and Applied Ecology, 12(1), 10-20.
- McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. Trends in ecology and evolution, 21(4), 178-185.
- McKinney, M. L. (2005). Species introduced from nearby sources have a more homogenizing effect than species from distant sources: evidence from plants and fishes in the USA. Diversity and Distributions, 11(5), 367-374.
- McKinney, M. L., and Lockwood, J. L. (1999). Biotic homogenization: a few winners replacing many losers in the next mass extinction. Trends in ecology and evolution, 14(11), 450-453.
- Mondoni, A., Rossi, G., Orsenigo, S., and Probert, R. J. (2012). Climate warming could shift the timing of seed germination in alpine plants. Annals of Botany, 110(1), 155-164.
- Mondoni, A., Pedrini, S., Bernareggi, G., Rossi, G., Abeli, T., Probert, R. J., ... and Orsenigo, S. (2015). Climate warming could increase recruitment success in glacier foreland plants. Annals of Botany, 116(6), 907-916.
- Moreno, C. E., and Rodríguez, P. (2010). A consistent terminology for quantifying species diversity?. Oecologia, 163(2), 279-282.
- Nagy, L., and Grabherr, G. (2009). The biology of alpine habitats. Oxford University Press.

- Neilson, R. P., Pitelka, L. F., Solomon, A. M., Nathan, R. A. N., Midgley, G. F., Fragoso, J. M., ... and Thompson, K. E. N. (2005). Forecasting regional to global plant migration in response to climate change. Bioscience, 55(9), 749-759.
- Nogués-Bravo, D., Araújo, M. B., Errea, M. P., and Martinez-Rica, J. P. (2007). Exposure of global mountain systems to climate warming during the 21st Century. Global Environmental Change, 17(3), 420-428.
- Nogués-Bravo, D. N., Araújo, M. B., Lasanta, T., and Moreno, J. I. L. (2008). Climate change in Mediterranean mountains during the 21st century. AMBIO: A Journal of the Human Environment, 37(4), 280-285.
- Olden, J. D., and Rooney, T. P. (2006). On defining and quantifying biotic homogenization. Global Ecology and Biogeography, 15(2), 113-120.
- Orsenigo, S., Mondoni, A., Rossi, G., and Abeli, T. (2014). Some like it hot and some like it cold, but not too much: plant responses to climate extremes. Plant Ecology, 215(7), 677-688.
- Osborn, T., and Jones, P. (2019). Global Temperature Record.
- Ozinga, W. A., Römermann, C., Bekker, R. M., Prinzing, A., Tamis, W. L., Schaminée, J. H., ... and Van Groenendael, J. M. (2009). Dispersal failure contributes to plant losses in NW Europe. Ecology Letters, 12(1), 66-74.
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. Annual Review of Ecology, Evolution, and Systematics, 637-669.
- Parolo, G., and Rossi, G. (2008). Upward migration of vascular plants following a climate warming trend in the Alps. Basic and Applied Ecology, 9(2), 100-107.
- Pauli, H., Gottfried, M., Hohenwallner, D., Hülber, K., Reiter, K., and Grabherr, G. (2001a). Gloria—the multi-summit approach. Field manual, 2nd draft version, Vienna.
- Pauli, H., Gottfried, M., and Grabherr, G. (2001b). High summits of the Alps in a changing climate. In “Fingerprints” of Climate Change (pp. 139-149). Springer, Boston, MA.
- Pauli, H., Gottfried, M., Hohenwallner, D., Reiter, K., Casale, R., and Grabherr, G. (2004). The GLORIA Field Manual-Multi-Summit Approach. European Commission, DG Research, EUR 21213. Official Publications of the European Communities.
- Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Alonso, J. L. B., ... and Grabherr, G. (2012). Recent plant diversity changes on Europe’s mountain summits. Science, 336(6079), 353-355.
- Pearson, R. G. (2006). Climate change and the migration capacity of species. Trends in Ecology and Evolution, 21(3), 111-113.

- Poschlod, P., Abedi, M., Bartelheimer, M., Drobnik, J., Rosbakh, S., and Saatkamp, A. (2013). Seed ecology and assembly rules in plant communities. Vegetation Ecology, 164-202.
- Rixen, C., Wipf, S., Frei, E., and Stöckli, V. (2014). Faster, higher, more? Past, present and future dynamics of alpine and arctic flora under climate change. Alpine Botany, 124(2), 77-79.
- Rixen, C., and Wipf, S. (2017). Non-equilibrium in alpine plant assemblages: shifts in Europe's summit floras. In High mountain conservation in a changing world (pp. 285-303). Springer, Cham.
- Rogora, M., Frate, L., Carranza, M. L., Freppaz, M., Stanisci, A., Bertani, I., ... and Cerrato, C. (2018). Assessment of climate change effects on mountain ecosystems through a cross-site analysis in the Alps and Apennines. Science of the total environment, 624, 1429-1442.
- Root, T. L., Price, J. T., Hall, K. R., Schneider, S. H., Rosenzweig, C., and Pounds, J. A. (2003). Fingerprints of global warming on wild animals and plants. Nature, 421(6918), 57-60.
- Root, T. L., MacMynowski, D. P., Mastrandrea, M. D., and Schneider, S. H. (2005). Human-modified temperatures induce species changes: joint attribution.Proceedings of the National Academy of Sciences of the United States of America, 102(21), 7465-7469.
- Rosbakh, S., Leingärtner, A., Hoiss, B., Krauss, J., Steffan-Dewenter, I., and Poschlod, P. (2017). Contrasting effects of extreme drought and snowmelt patterns on mountain plants along an elevation gradient. Frontiers in plant science, 8, 1478.
- Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q., Casassa, G., ... and Imeson, A. (2008). Attributing physical and biological impacts to anthropogenic climate change. Nature, 453(7193), 353-357.
- Ross, L. C., Woodin, S. J., Hester, A. J., Thompson, D. B., and Birks, H. J. B. (2012). Biotic homogenization of upland vegetation: patterns and drivers at multiple spatial scales over five decades. Journal of vegetation science, 23(4), 755-770.
- Rossi, G., and Parolo, G. (2005). Gli effetti dei cambiamenti climatici sulle specie vascolari degli ambienti di alta quota: i casi-studio delle Alpi Retiche e dell‟ Appennino settentrionale. Informatore Botanico Italiano, 37(1), 238-239.
- Rustad, L. E. J. L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., ... and Gurevitch, J. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia, 126(4), 543-562.

- Schmid, G. L. (2004). The role of soils in recording environmental change at alpine treeline in Glacier National Park, Montana. Texas State University-San Marcos.
- Schär, C., Vidale, P. L., Lüthi, D., Frei, C., Häberli, C., Liniger, M. A., and Appenzeller, C. (2004). The role of increasing temperature variability in European summer heatwaves. Nature, 427(6972), 332.
- Shannon, C. E., and Weaver, W. (1948). The mathematical theory of communication. Urbana, IL.
- Shimono, Y., and Kudo, G. (2005). Comparisons of germination traits of alpine plants between fellfield and snowbed habitats. Ecological Research, 20(2), 189-197.
- Silvertown, J., and Charlesworth, D. (2001). Introduction to plant population biology, 4th edn Oxford, Wiley-Blackwell.
- Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K. B., Tignor, M. and Miller, H. L. (2007). Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge, UK: Cambridge University Press.
- Sørensen, T. (1948). {A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons}. Biol. skr., 5, 1-34.
- Steinbauer, M. J., Grytnes, J. A., Jurasinski, G., Kulonen, A., Lenoir, J., Pauli, H., ... and Bjorkman, A. D. (2018). Accelerated increase in plant species richness on mountain summits is linked to warming. Nature, 556(7700), 231.
- Thackeray, S. J., Sparks, T. H., Frederiksen, M., Burthe, S., Bacon, P. J., Bell, J. R., ... and Wanless, S. (2010). Trophic level asynchrony in rates of phenological change for marine, freshwater and terrestrial environments. Global Change Biology, 16(12), 3304-3313.
- Theurillat, J. P., and Guisan, A. (2001). Potential impact of climate change on vegetation in the European Alps: a review. Climatic Change, 50(1-2), 77-109.
- Thuiller, W., Lavorel, S., Araujo, M.B., Sykes, M.T., Colin Prentice. (2005). Climate change threats to plant diversity in Europe. PNAS, 102 (23), 8245-8250.
- Tudela-Isanta, M., Fernández-Pascual, E., Wijayasinghe, M., Orsenigo, S., Rossi, G., Pritchard, H. W., and Mondoni, A. (2018a). Habitat-related seed germination traits in alpine habitats. Ecology and Evolution, 8(1), 150-161.
- Tudela-Isanta, M., Ladouceur, E., Wijayasinghe, M., Pritchard, H. W., and Mondoni, A. (2018b). The seed germination niche limits the distribution of some plant species in calcareous or siliceous alpine bedrocks. Alpine Botany, 128(1), 83-95.

- Tuomisto, H. (2010a). A diversity of beta diversities: straightening up a concept gone awry. Part 1. Defining beta diversity as a function of alpha and gamma diversity. Ecography, 33(1), 2-22.
- Tuomisto, H. (2010b). A diversity of beta diversities: straightening up a concept gone awry. Part 2. Quantifying beta diversity and related phenomena. Ecography, 33(1), 23-45.
- Tuomisto, H. (2010c). A consistent terminology for quantifying species diversity? Yes, it does exist. Oecologia, 164(4), 853-860.
- Väre, H., Lampinen, R., Humphries, C., and Williams, P. (2003). A Europe-Wide Assessment of Biological Richness and Change. in Alpine Biodiversity in Europe, Springer, 133-148
- Vellend, M. (2001). Do commonly used indices of β-diversity measure species turnover?. Journal of Vegetation Science, 12(4), 545-552.
- Venn, S., Pickering, C., and Green, K. (2012). Short-term variation in species richness across an altitudinal gradient of alpine summits. Biodiversity and Conservation, 21(12), 3157-3186.
- Vittoz, P., Bodin, J., Ungricht, S., Burga, C. A., and Walther, G. R. (2008). One century of vegetation change on Isla Persa, a nunatak in the Bernina massif in the Swiss Alps. Journal of Vegetation Science, 19(5), 671-680.
- Vittoz, P., Bayfield, N., Brooker, R., Elston, D. A., Duff, E. I., Theurillat, J. P., and Guisan, A. (2010). Reproducibility of species lists, visual cover estimates and frequency methods for recording high-mountain vegetation. Journal of Vegetation Science, 21(6), 1035-1047.
- Voigt, T., Füssel, H. M., Gärtner-Roer, I., Huggel, C., Marty, C., and Zemp, M. (2010). Impacts of climate change on snow, ice, and permafrost in Europe: observed trends, future projections, and socio-economic relevance. European Topic Centre on Air and Climate Change.
- Walter, H. (1984). Vegetation und Klimazonen. Stuttgart, Ulmer.
- Walter, H. (1985). Vegetation of the earth and ecological systems of the geo-biosphere. Springer-Verlag. Berlin, Germany.
- Walter, H., and Breckle, S. W. (1991). Okologische Grundlagen in globaler Sicht. Fischer.
- Breckle, S. W., and Walter, H. (2002). Walter's vegetation of the earth: the ecological systems of the geo-biosphere. Springer.
- Walther, G. R., Beißner, S., and Burga, C. A. (2005). Trends in the upward shift of alpine plants. Journal of Vegetation Science, 16(5), 541-548.
- Walther, G. R., Roques, A., Hulme, P. E., Sykes, M. T., Pyšek, P., Kühn, I., ... and Settele, J. (2009). Alien species in a warmer world: risks and opportunities. Trends in Ecology and Evolution, 24(12), 686-693.

- Wahid, A., Gelani, S., Ashraf, M., and Foolad, M. R. (2007). Heat tolerance in plants: an overview. Environmental and Experimental Botany, 61(3), 199-223.
- Walck, J. L., Baskin, J. M., and Baskin, C. C. (1997). A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of Solidago (Asteraceae). 1. Germination phenology and effect of cold stratification on germination. Seed Science Research, 7(1), 47-58.
- Walck, J., and Dixon, K. (2009). Time to future-proof plants in storage. Nature, 462(7274), 721.
- Walck, J. L., Hidayati, S. N., Dixon, K. W., Thompson, K. E. N., and Poschlod, P. (2011). Climate change and plant regeneration from seed. Global Change Biology, 17(6), 2145-2161.
- Warren, R., Van Der Wal, J., Price, J., Welbergen, J. A., Atkinson, I., Ramirez-Villegas, J., ... and Lowe, J. (2013). Quantifying the benefit of early climate change mitigation in avoiding biodiversity loss. Nature Climate Change, 3(7), 678.
- Welling, P., and Laine, K. (2002). Regeneration by seeds in alpine meadow and heath vegetation in sub-arctic Finland. Journal of Vegetation Science, 13(2), 217-226.
- Whittaker, R. H. (1960). Vegetation of the Siskiyou mountains, Oregon and California. Ecological Monographs, 30(3), 279-338.
- Wilson, M. V., and Shmida, A. (1984). Measuring beta diversity with presence-absence data. The Journal of Ecology, 1055-1064.
- Wipf, S., Stöckli, V., Herz, K., and Rixen, C. (2013). The oldest monitoring site of the Alps revisited: accelerated increase in plant species richness on Piz Linard summit since 1835. Plant Ecology and Diversity, 6(3-4), 447-455.
- Woodward, F. Ian, and B. G. Williams (1987). Climate and plant distribution at global and local scales. Vegetatio 69.1-3: 189-197.
- Zemp, M., Haeberli, W., Hoelzle, M., and Paul, F. (2006). Alpine glaciers to disappear within decades?. Geophysical Research Letters, 33(13).
- Official GLORIA web site (http://www.gloria.ac.at/?a=2)

Article I

Porro, F., Tomaselli, M., Abeli, T., Gandini, M., Gualmini, M., Orsenigo, S., ... and Carbognani, M. (2019). Could plant diversity metrics explain climate-driven vegetation changes on mountain summits of the GLORIA network? Biodiversity and Conservation, 1-22.

Supplementary material is provided after the references section: pp 58-61

2. Could plant diversity metrics explain climate-driven vegetation changes on mountain summits of the GLORIA network?

2.1 Abstract

High-elevation habitats host a large number of plant species and are characterized by high biodiversity. The vegetation dynamics in these cold adapted ecosystems are difficult to predict, being affected by global warming, especially in the last decades. With the aim to promote a better understanding of climate-driven changes of alpine vegetation, we investigated the variation in species richness, α-diversity, β-diversity, and total cover of plant functional types over a time lapse of 15 years, relying on multiple re-surveys of mountain summit vegetation in 2001, 2008 and 2015. The study area, included in the long-term global observation network GLORIA, was at the boundary between temperate and Mediterranean mountains of S-Europe (northern Apennines, Italy). We identified a trend of loss in biodiversity and signals of biotic homogenization using multiple diversity metrics, despite the overall species richness increment observed in the study area. Cold-adapted and rare species declined while dominant species like graminoids and shrubs increased. Our results highlight that long-term vegetation monitoring activities paired with multiple measures of diversity are required to properly assess biodiversity and to obtain useful indications for future conservation activities in alpine environments. The methods here presented could be applied in all GLORIA sites to quantify biodiversity changes over time, obtaining comparable results for biodiversity monitoring in high-elevation habitats from all over the world.

Keywords

GLORIA, biodiversity loss, vegetation dynamics, biotic homogenization, plant functional types, Hill numbers

2.2 Introduction

Mountains are regarded as biodiversity hotspots (Körner et al. 2017) and are experiencing higher rates of climate change than other regions of the world (IPCC 2014; Mountain Research Initiative EDW Working Group 2015). In particular, alpine ecosystems are especially sensitive to global warming, as biological and chemical processes are more temperature-sensitive in cold environments (Kirschbaum 1995; Körner 1995).

As reported by several studies (Grabherr et al. 1994; Walther et al. 2005; Rixen et al. 2014), the observed climate warming has directly affected alpine plant communities, causing changes in distribution, physiology and phenology of a wide range of taxa, and promoting both species richness increase and cryophilic species extinction (e.g. Sala et al. 2000; Parmesan 2006; Bellard et al. 2012; Pauli et al. 2014; Steinbauer et al. 2018). These concerns have been confirmed by recent studies, indicating that variation in temperature and precipitation along with extreme climatic events (e.g. heat waves) represent one of the major threats for biodiversity of the alpine ecosystems (Abeli et al. 2012a; Ernakovich et al. 2014; Orsenigo et al. 2014).

Increased temperatures result in direct abiotic pressure for resident alpine species by altering their physiology and phenology (Fu et al. 2015; Carbognani et al. 2016), and, indirectly, the rise of temperatures is opening "new ground" for invaders from lower elevations. This process, known as upward migration, is causing an increase in species richness in alpine and other cold environments (Grabherr et al. 1994; Walther et al. 2005, Steinbauer et al. 2018), with predicted local extinctions of those plants that already live close to the upper margins of elevational gradients (Theurillat and Guisan 2001; Colwell et al. 2008; Pauli et al. 2012).

Resurvey of historical sites and long-term monitoring studies provided evidences of the occurring vegetation changes at local and regional scale, reporting upward shifts of plant species often associated with an increase in species richness (Parolo and Rossi 2008; Lenoir et al. 2008; Steinbauer et al. 2018). According to the data collected in 2001 and 2008 following the sampling design proposed by the long-term monitoring project GLORIA (Global Observation Research Initiative in Alpine environments; https://gloria.ac.at/) on 66 different mountain peaks located all over Europe, species gains seemed to be particularly evident in boreal and temperate European mountains, whereas most of the Mediterranean summits had, in contrast, species losses (Pauli et al. 2012). Both gains and losses were more pronounced on lower summits, suggesting that changes in species richness are mostly driven by leading edge expansions in boreal and temperate mountains and by rear edge retractions in the Mediterranean ones (Pauli et al. 2012). Considering that rear edges retract as much as leading edges expand (Rumpf et al. 2019), these regional-specific patterns suggest that different processes could drive the vegetation dynamics in different mountain regions. However, it is not known if these trends will remain constant over longer time-scales, in particular in mountain ranges straddling different climatic regimes.

Dullinger et al. (2012) introduced a model indicating that the opposing effects of delayed local population extinctions and lagged migration rates will result in less severe twenty-first-century range reductions of alpine plants than expected from static, niche-based model predictions, and provided insights into the transient dynamics that are likely to dominate the range responses of plants to climate warming over the next century. In addition, Alexander et al. (2018) developed a process-based and dynamic community model to understand how dispersal lags and competitive interactions could influence expectations for range shifts and rates of community turnover along elevation under climate change.

Up to now, many studies on the impact of changing climate on plant diversity in mountain summits were focused on species richness (see Stanisci et al. 2005; Walther et al. 2005; Holzinger et al. 2008; Pauli et al. 2012; Wipf et al. 2013; Steinbauer et al. 2018). Nonetheless, species richness alone is a weak indicator as it does not take into account the abundance of species, which is another fundamental component of the biological diversity of plant assemblages. Also within the GLORIA network, a comprehensive review on diversity changes capable of going beyond species richness is still missing. Diversity is a complex multidimensional property of a vegetation stand and many indices have been developed to quantify α-diversity. However, methods integrating the sensitivity of diversity estimates to species abundances, such as the diversity profiles based on Hill numbers (Hill 1973), remain currently underused in plant ecological research. Hill numbers are a parametric family of diversity indices and have several advantages over other single diversity metrics (Chao et al. 2014). They are expressed by a clear unit of measure (*effective number of species*) and can depict simultaneously the values of several diversity indices with different sensitivity to species richness and evenness. As such, they could provide new insight for comparing and interpreting vegetation diversity.

Among the plant functional types, forbs are the major contributor to species diversity in alpine and arctic communities (Körner 1995). However, shrubs and graminoids are commonly regarded as the most responsive groups to warming in cold ecosystems (Bigelow et al. 2003, Elmendorf et al. 2012; Stanisci et al. 2016). Nevertheless, the influence of climate change on plant functional types seems region-specific (Elmendorf et al. 2012). For instance, warming trends lead to shrubification in tundra ecosystems (e.g. Bigelow et al. 2003; Myers-Smith et al. 2011; Myers-Smith et al. 2015) with a shift from herbaceous to woody communities (e.g. Walker et al. 2006). Differently, in less extreme habitats, shrubs can be outcompeted by graminoids (Thompson et al. 1995), probably due to the greater response of graminoids to soil nutrient availability (Dormann and Woodin 2002). Soil nitrogen concentration in natural environmental is related to temperature (Rustad et al. 2001), it is constantly increasing in alpine and subalpine environments (Rogora et al. 2006), and it has already been recognized as a co-driver for species composition changes, often associated with increments in nitrophilous species abundance and biodiversity loss (Bobbink et al. 2010). Consequently, our knowledge of warming-induced vegetation dynamics in mountain areas at the intersection of different climatic regimes and their consequences for plant diversity are still incomplete.To fully describe the processes that are taking place in plant communities, scientific investigations regarding the influence of global change on alpine ecosystems should include variation of both α- and β-diversity, since the increase of α-diversity on mountain summits can be accompanied by decreasing β-diversity, leading to a vegetation homogenization over time (Jurasinski and Kreyling 2007; Britton et al. 2009; Ross et al. 2012).

In this context, we propose here a multi-faceted approach to analyse the changes in vascular plant diversity (using multiple α- and β-diversity measures) and cover of the main functional types (forbs, graminoids, and shrubs) recorded through multiple re-surveys. The proposed methodology could be used in other long-term vegetation monitoring studies, especially within the global observation network GLORIA.

The methods proposed here have been used to assess the diversity changes occurred in the IT-NAP GLORIA Target Region corresponding to the northern Apennines, a mountain system lying at the boundary between northern and central Italy. The spatial position of the northern Apennines is important from a phytogeographical point of view, as it stands at the southernmost boundary of the temperate region and at the forefront of the Mediterranean region. In 2008, this Target Region shared the same general increasing trend in species richness recorded in other boreal and temperate Target Regions within the European GLORIA network. In contrast, the Central Apennines Target Region (about 350 km southeast) and the other Mediterranean Regions showed a different trend of species richness remaining stable or decreasing (Pauli et al. 2012; Stanisci et al. 2016).

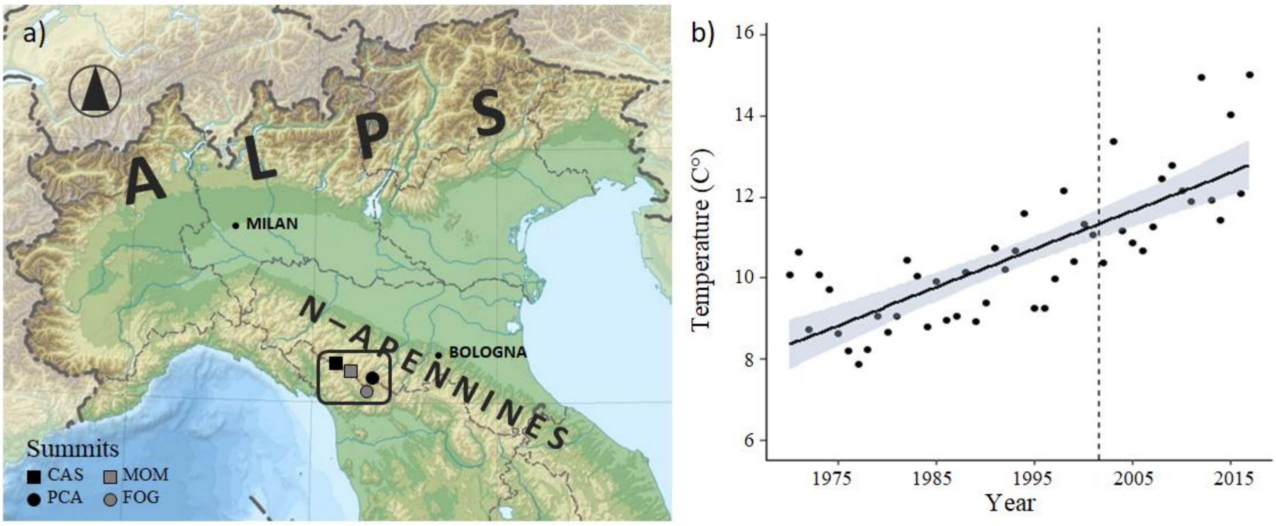
In particular, applying the methods presented in this paper, we aimed to address the following questions: (1) Did changes in species richness from 2001 to 2015 confirm the magnitude and the “temperate-boreal” trend of changes observed in the period 2001-2008? (2) Did α-diversity vary between 2001 and 2015 and, if so, were the changes consistent among different summits? (3) Did the cover of plant functional types vary over time? (4) Was the shift in species composition leading to a homogenization and biodiversity loss of the mountain summits?

2.3 Materials and Methods

Study area

In 2001, four summits were selected in the GLORIA Target Region "IT-NAP" (Fig. 1a), following the multi-summit approach proposed by Pauli et al. (2015). The four summits, Mt. Casarola, Alpe di Mommio, Cima di Pian Cavallaro and Cima di Foce a Giovo, will be hereafter labelled respectively as CAS, MOM, PCA and FOG. Long-term climatic features of the four summits, along with geographical information, are reported in Table S1 in Appendix. The four study sites, located on the top of the four summits, lay above the treeline, which stands at around 1700 m a.s.l. (Tomaselli and Rossi, 1994), and are disposed along an elevational gradient that ranges from 1722 m to 1978 m. The summits were selected to represent the vegetation patterns occurring in the summit areas of the Target Region according to the requirements reported in the GLORIA field manual (Pauli et al. 2015). Moreover, all the sampled areas were in the recent past and are also presently free from grazing or other anthropogenic impacts. Geologically, the summits consist of thick-bedded turbiditic sandstones and marlstones corresponding to the Macigno formation (Dallan Nardi and Nardi 1974). Climatic data recorded by the Meteorological Observatory of Mt. Cimone, located about 1 km from PCA summit, indicates a significant warming trend of summer air temperatures for the period 1970-2017 (as shown by the linear regression model fitted to the summer temperature data against years; R^2 = 0.613, t = 8.539, $F_{1\text{-}46}$ = 72.92, p <0.001) with an average increment of 0.094 K year^{-1} (Fig. 1b).

Fig. 1 a) Schematic map of the study area with geographic position of the sampled summits. CAS: Mt. Casarola; MOM: Alpe di Mommio; PCA: Cima di Pian Cavallaro; FOG: Cima di Foce a Giovo. **b)** Average summer temperatures (June to August) recorded at the Meteorological Observatory at Mt. Cimone (northern Apennines) from 1970 to 2017. Starting year of the GLORIA monitoring activity is highlighted by the dotted line (slope = 0.094, R^2 = 0.613, p <0.001).



In the study area, summit vegetation consists of dwarf shrub heaths dominated by *Vaccinium* species, mostly occurring on the northern slopes, and of secondary grasslands dominated by *Brachypodium genuense* or by *Nardus stricta*, prevailing over the southern slope (Tomaselli 1994; Ferrari and Piccoli 1997).

Field work

Three vegetation surveys were carried out in the summer of 2001, 2008 and 2015, following the sampling design of GLORIA (Pauli et al. 2015; https://gloria.ac.at/downloads/manual). At each summit of the Target Region the area investigated was defined as a polygon with four corners fixed at each cardinal direction, placed 10 vertical metres below the highest summit point (HSP) of the summit, and delimited by a perimeter called 10m contour line. Each summit's polygon was then divided into four sectors, following cardinal directions facing N, S, E and W. Each sector was further subdivided into 0-5 and 5-10 m zones below the HSP, by a line called 5m contour line, obtaining eight summit area sections (referred as "SAS" hereafter) for each summit. The group of four SAS lying above the 5m contour line and group of four SAS lying below it will be referred hereafter as "upper belt" and "lower belt". In total, 32 SAS were included in the Target Region. For each SAS, a complete list of vascular plant species was recorded, along with a visually estimated coverage expressed by the following classes of abundance: r! = very rare, r = rare, s = scattered, c = common, d = dominant, following GLORIA 2015 guidelines (Pauli et al. 2015) (see Table S2 in Appendix). Species nomenclature follows Tutin et al. (1964-1993). Species richness values recorded during the three surveys for the Target Region, summit and belt levels are reported in Table S3. The area of each SAS was estimated by trigonometry on the basis of angles and lengths measured in the field (see Table S4 in Appendix for SAS polygon area).

Data processing

First, to avoid pseudo-absence or pseudo-presence signals, following the indications of Vittoz et al. (2010a) and Pauli et al. (2012), all the species that were found only in one SAS and just once among the three surveys were excluded from the original species list (14 species, see Table S5 in Appendix). Potential mistakes in species identifications, or changes in nomenclature, were corrected by a scrupulous check of possible synonymies and by aggregating the records of pairs of species with frequent confusions into the same taxon. Second, the five qualitative abundance classes defined by the GLORIA protocol were converted to cover percentage approximations, on the basis of the GLORIA class descriptors and their percentage breakpoints. The values used for the conversion were assigned using the cover percentage approximations (e.g. the mid values of the Braun-Blanquet cover/abundance scale transformation) used for the numerical treatment of phytosociological data (Braun-Blanquet 1964; Van der Maarel 1979). Converted values are reported in Table S2 in Appendix. Finally, to consider the differences in SAS areas due to summit micro-topography, species cover was standardized by means of a correction factor determined by the ratio between the SAS area where a species occurred and the cumulative SAS area.

Species richness

To detect if species richness varied through time at the Target Region level, taking into account the species richness – area relationship, we firstly calculated the residuals of a linear mixed-effect model (LMM) fitted on the 32 SAS species richness values in 2001, 2008 and 2015, with “SAS area” as fixed effect. To taking into account the repeated measures and the spatial structure of the SASs, random intercepts for the nested factors SAS ID and Summit ID were included in the model. Subsequently, to eliminate the effect of SAS area on species richness, we fitted a linear model (LM) on the obtained LMM residuals, similarly to the approach proposed by Vittoz et al. (2010b). The LM was performed including “Time” (3-level factor: 2001, 2008 and 2015), “Summit” (4-level factor: CAS, MOM, PCA,FOG), and “Belt” (2-level factor: upper and lower belts) as fixed effect, to test if the species richness changed over time in the Target Region, and if there were differences among the summits and belts. The interactions among Time, Summit and Belt were also included to test if species richness changes were consistent over the Target Region. We choose this approach as the different abiotic conditions and vegetation of the 4 summits and belts could have had different effects on the species richness changes (e.g. we did expect higher rates of new species recruitment in the lower summits and SAS belts, as a consequence of upward migration). The same approach was then repeated at summit level (8 SAS for each summit), calculating with a LMM the SAS species richness residuals on summits’ SAS areas (with “SAS ID” as a random factor), and then by fitting a LM on the obtained residuals with “Time”, “Belt” and their interaction term as fixed factors. LMM and LM were performed by means of the packages lme4 (Bates et al. 2015), F-statistics and *p*-values were obtained using the function “Anova” by means of “car” package (Fox et al. 2012), while Post Hoc tests were performed with the “TukeyHSD” function of the package “stats” of the statistical suite R version 3.5.2 (R Core Team 2018).

Finally, plant species that disappeared entirely (i.e. species sampled in 2001, and then not found again) from the Target Region, or in at least one summit, were included in two lists that could be useful for plant conservation activities.

Assessment of α-diversity

To assess α-diversity changes, we calculated the diversity profiles in 2001 and 2015 for the whole Target Region, as well as for the four summits separately. The diversity profiles were obtained by calculating the Hill Numbers (^qD *sensu* Hill 1973) of each assemblage, using the SAS standardized cover of species. Hill numbers have been recently re-introduced into the analyses of ecological data by Jost (2006), as they have the possibility to describe different aspects of diversity of one site at once, allowing to rank different assemblages from high to low diversity (Tóthmérész 1998; Chao et al. 2014). Hill numbers are a mathematically unified family of diversity indices expressed in units of *effective numbers of species* (i.e. the number of equally abundant species that would be needed to give the same value of a diversity measure; Hill, 1973), calculated from the frequencies of each component species, and a scale parameter, defined as:

$$^qD = \left(\sum_{i=1}^{S} p_i^q\right)^{1/(1-q)}$$

where p_i is the relative frequency of the ith species, S is the total number of species and q is the scale parameter.

This last parameter determines the index sensitivity to species relative abundance: at small values of q, Hill numbers are less influenced by species abundance (being ^{q}D values at $q = 0$ equal to species richness), while as q increases, their values are increasingly influenced by the abundance of species. The ^qD values of an assemblage can be plotted against the scale parameter to obtain a decreasing function, called diversity profile (Patil and Taillie 1979, 1982). A diversity profile is composed by several diversity indices that have different sensitivity to the presence of rare and abundant species and using them as a tool to evaluate assemblages' diversity allows us to overcome the limits of a single numerical definition. The value of a single index cannot adequately summarize biodiversity and community structure, as it is well-known that different diversity indices may rank sets of communities in different ways (Pielou 1975), depending on how it is influenced by species abundance (Hill 1973). For this reason, diversity profiles can be used to compare the diversity of different sites consistently (or, as in this study, to compare diversity of the same assemblage in different years). If two diversity profiles do not intersect each other, the assemblage with the diversity profile that runs above the other one is unequivocally more diverse. However, if the profiles cross each other, the two assemblages cannot be unequivocally ordered, as one assemblage is more diverse for the rare species while another is more diverse for the frequent species (Tóthmérész, 1998). In order to rank diversity profiles even when they cross each other, we calculated the underlying area of the diversity profile function, that will be referred as "surface area" hereafter, as suggested by Di Battista et al. (2017), using the trapezoid method (Atkinson 2008). Surface area is directly proportional to both species evenness and species richness, and it does not attribute more importance to one or another aspect, as it is calculated while considering the entire domain. From an ecological point of view, a higher value of the surface area denotes a greater community biodiversity. To gather further information for interpreting diversity variation, we calculated the following indices: (1) Shannon index (Shannon 1948), (2) Sheldon evenness (Sheldon 1969), and (3) Dominance index, expressed as 1 – Simpson index (Simpson 1949). Diversity profiles and indices were calculated and plotted with the software PAST 3.21 (Hammer et al. 2001).

Cover changes of plant functional types

To analyse vegetation dynamics over time, we grouped the species in the following plant functional types classified by growth forms: forbs, graminoids (including grasses, sedges and rushes), and shrubs. This simplified classification is currently adopted in the studies concerning predictions of vegetation responses to global change in Arctic and boreal ecosystems (see Chapin et al. 1996; Wullschleger et al. 2014; Thomas et al. 2019). Ferns were excluded from this analysis due to their low abundance on the summits investigated. For each functional type, we calculated the sum of standardized cover of all species occurring in each SAS at the Target Region level (32 SAS included). To assess if significant differences in the cover of functional types occurred between 2001 and 2015, we fitted LMMs to log-transformed data by means of the lme4 package (Bates et al. 2014), with "Time" (2-level factor: 2001 and 2015) as fixed effect and "Summit ID" and "SAS ID" as nested random effect, in order to take into account both spatial and temporal relationship within the data. For the functional types showing significant variation over time, further LMMs were performed at the summit level (including 8 SAS each). In these models the log-transformed standardized cover of functional types was the response variable, whereas "Time" and "SAS ID" were the fixed and random effect, respectively. F-statistics and *p*-values were obtained using the function "Anova" by means of "car" package (Fox et al. 2012).

Assessment of β-diversity

To evaluate if a process of biotic homogenization is currently ongoing in the study area, we calculated β-diversity for 2001 and 2015, as suggested in other researches (Jurasinski and Kreyling 2007; Britton et al. 2009; Ross et al. 2012). First, we used the dissimilarity among all the SAS of the Target Region as a descriptor of the spatial patterns of diversity. The Sørensen multiple-site dissimilarity measure described in Baselga (2010) was calculated for the Target Region level (32 SAS included) for 2001 and 2015. This measure is dependent on the proportion of species shared between different assemblages, and it is related to species turnover, nestedness and heterogeneity (Koleff et al. 2003; Baselga 2010). This measure can be partitioned in the two separate indices "multiple-site β_{SIM}" and "multiple-site β_{SNE}" (accounting for the dissimilarity among sites due to turnover and nestedness, respectively), and it is specifically designed to quantify the overall heterogeneity of a pool of sites (Baselga 2013). In particular, we focused on the changes of the "multiple-site β_{SIM}", the multiple-site version of the Simpson β-diversity index (*sensu* Baselga 2010), which is regarded as one of the β-diversity indices that perform better under different conditions and is less biased by sample size and nestedness of the quadrant (Koleff 2003; Barwell 2015). In addition, we calculated the "pairwise β_{sim}" index (*sensu* Baselga 2010) between each SAS within the same period, testing differences between 2001 and 2015 with the Wilcoxon test for paired data. Finally, these analyses were repeated for the upper and lower belts (including 16 SAS each) to understand if there are differences in terms of β-diversity due to upward migration and species extinction. β-diversity analyses were performed by means of the "Betapart" (Baselga and Orme 2012) and "PairedData" packages (Champely 2018) of the statistical environment R version 3.5.2.

2.4 Results

Species richness

In the three surveys, a total of 169 vascular plant species were recorded at least once in the study area. The number of vascular plants in the Target Region varied over time (Table S3), as testified by the significant effect of the factor Time on species richness (Table 1a). In particular, the number of species recorded in each SAS increased significantly from 2001 to 2008 (mean difference ± 95% CI: +4.1 ± 1.83, $p < 0.001$) and to 2015 (+4.3 ± 1.83, $p < 0.001$), whereas the species richness recorded during the two later surveys was not significantly different (+0.15 ± 1.83, $p = 0.977$). The number of colonizers decreased from 11 species between 2001 and 2008 to 3 species between 2008 and 2015, whereas disappearances increased from 1 to 8.

Table 1 Results of the linear models for species richness in relation to Time, Summit and Belt for: a) Target Region (32 SAS), b) summits (8 SAS for each of the four summits: CAS, MOM, PCA and FOG); Degrees of freedom (Df), F statistic and significance level are shown. *p*-values below 0.05 are given in bold (the analysis were run on the residual of species richness versus SAS area).

Scale level	Factor	Df	F	*p*-value
a) Target Region				
	Time	2	20.07	**< 0.001**
	Summit	3	0.094	0.96
	Belt	1	2.25	0.14
	Time × Summit	6	8.32	**< 0.001**
	Time × Belt	2	0.36	0.70
	Time × Summit × Belt	6	1.74	0.12
	Summit × Belt	3	0.13	0.94
b) summits				
CAS	Time	2	0.18	0.83
	Belt	1	0.05	0.82
	Time × Belt	2	1.28	0.30
MOM	Time	2	2.09	0.15
	Belt	1	0.14	0.71
	Time × Belt	2	0.45	0.64
PCA	Time	2	31.63	**< 0.001**
	Belt	1	4.49	**0.049**
	Time × Belt	2	2.71	0.09
FOG	Time	2	10.69	**< 0.001**
	Belt	1	1.82	0.19
	Time × Belt	2	0.31	0.73

Species richness variation over time was not consistent among the four summits (significant Time × Summit interaction term in Table 1a). In particular, the effect of "Time" on Species Richness was significant for both the two lower summits PCA and FOG (mean difference: +12.12 ±4.06, $p < 0.001$, and +5.12 ±3.63, $p = 0.005$, respectively), whereas it had no significant effect in the two higher summits: CAS (+0.25 ±4.98, $p = 0.99$) and in MOM (−0.37 ±3.19, $p = 0.95$; see Table 1b).

Assessment of α-diversity

The Shannon index and the Sheldon evenness values observed in 2015 in the Target Region decreased compared to 2001, whereas the value of the Dominance index increased (Table 2a). The diversity profiles of the Target Region were very similar in 2001 and 2015 (Fig. 2) and the pronounced decline of the curves indicated an uneven distribution of relative abundances among plant species, in particular in 2015. However, the two profiles did intersect, thus an immediate ranking of diversity level could not be performed. On the other hand, focussing on the variation of the Surface area underlying the diversity profiles in 2001 and 2015, it could be stated that, despite the species richness increment in 2015, the overall diversity of the Target Region decreased (Fig. 2 and Table 2a).

Two different patterns of diversity profiles were observed within the four summits investigated (Fig. 3): (1) an overall diversity loss was detected in the two higher summits (CAS and MOM) along with stagnating species richness and (2) diversity variations (both increments and decrements, depending on the considered summit) accompanied by an increase in species richness were observed in the two lower summits (PCA and FOG). In particular, for the two higher summits the diversity profiles and surface area values in 2015 were lower to the 2001 ones (Figs. 3a, 3b and Table 2b), indicating diversity loss. Moreover, both Shannon index and Sheldon evenness calculated for these two summits decreased over the years, whereas the Dominance index increased (Table 2b). With respect to the two lower summits, PCA showed higher Hill numbers in 2015 for all values of the scale parameter *q* (Fig. 3c) and consistently, Shannon index, Sheldon evenness and Surface area increased indicating a diversity increment, while the Dominance index decreased (Table 2b). In FOG, despite the increase in species richness over time (Table 1b), diversity decreased in 2015 and thus the profiles crossed each other (Fig. 3d). In this summit, the Shannon index remained unchanged, but the decreasing Sheldon evenness and Surface area, along with increasing Dominance index, indicated an overall diversity loss.

Fig. 2 Diversity profiles obtained by calculating the Hill Numbers for the Target Region using the relative species cover percentage dataset of 2001 and 2015.

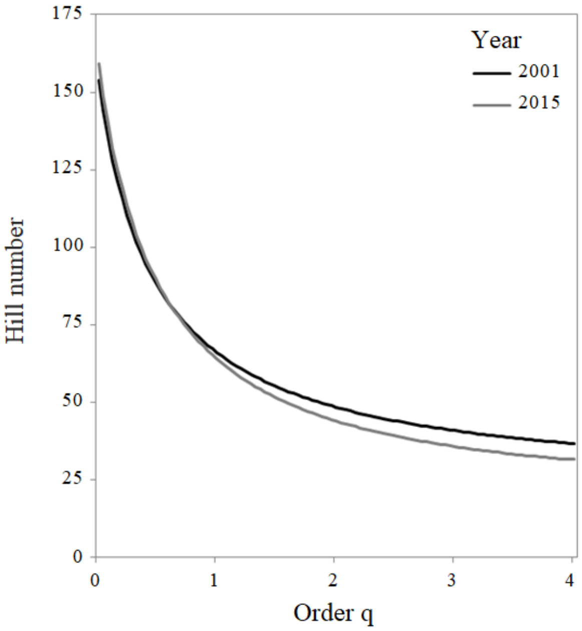


Fig. 3 Diversity profiles obtained by calculating the Hill Numbers for the four summits investigated (a: CAS, b: MOM, c: PCA and d: FOG) using the relative species cover percentage dataset of 2001 and 2015.

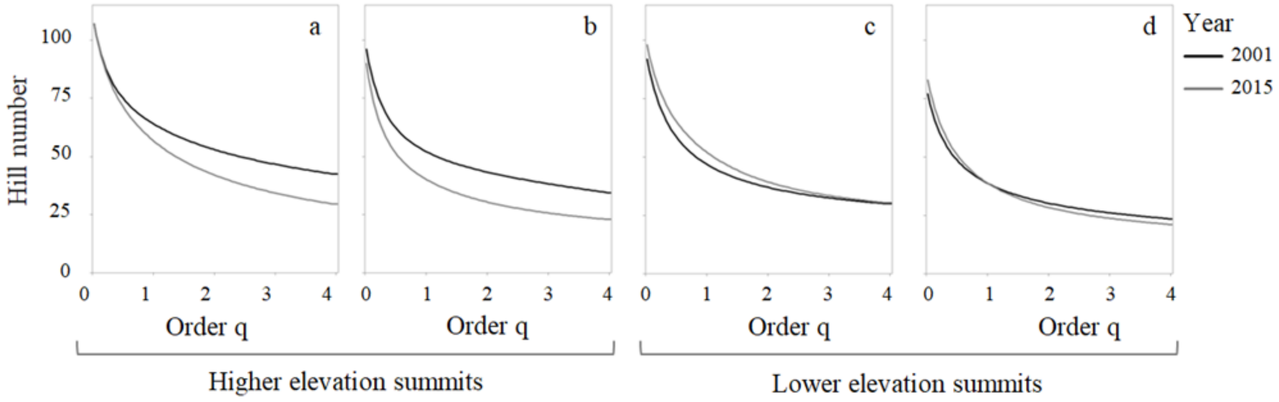


Table 2 Shannon diversity, Sheldon evenness and Dominance index values calculated for 2001 and 2015 at two different levels: a) Target Region (32 SAS), b) summits (8 SAS for each of the four summits: CAS, MOM, PCA and FOG). Surface area represents the underlying area of the diversity profile functions (see Figs. 2, 3).

Scale level	**Shannon index**		**Sheldon evenness**		**Dominance index**		**Surface area**	
	2001	2015	2001	2015	2001	2015	2001	2015
a) Target Region	4.19	4.16	0.43	0.40	0.022	0.025	231.1	218.8
b) summits								
CAS	4.15	4.03	0.59	0.53	0.019	0.026	227.2	190.0
MOM	3.95	3.69	0.54	0.44	0.025	0.033	187.7	140.3
PCA	3.84	3.94	0.50	0.53	0.028	0.027	166.6	178.6
FOG	3.65	3.65	0.50	0.46	0.032	0.036	136.3	133.2

Cover changes of plant functional types

The total cover of graminoids and shrubs increased between 2001 and 2015 at the Target Region level, showing however different patterns among the four summits studied (Table 3a). Conversely, forbs did not show any significant variation in space or over time. The magnitude of changes observed for graminoids was higher in comparison with the ones observed for shrubs at the Target Region level (Fig. 4). At the summit level, graminoids cover increased significantly on the two higher summits (Fig. 5, Table 3b), but no significant differences were detected on the lower summits. Shrubs and forbs did not show any significant change at the summit level.

Table 3 Results of linear mixed-effect models performed on the sum of standardized cover of plant functional types in relation to Time for: a) Target Region (32 SAS included) and b) summits (8 SAS included for each of the four summits: CAS, MOM, PCA and FOG). Degrees of freedom (Df), F statistic and significance level are shown. *p*-values below 0.05 are given in bold.

Scale level	Functional type	Factor	Df	F	*p*-value
a) Target Region					
	Forbs	Time	1-31	0.59	0.448
	Graminoids	Time	1-31	5.84	**0.022**
	Shrubs	Time	1-31	4.40	**0.044**
b) summits					
CAS	Graminoids	Time	1-7	11.06	**0.013**
	Shrubs	Time	1-7	1.08	0.334
MOM	Graminoids	Time	1-7	36.42	**< 0.001**
	Shrubs	Time	1-7	3.36	0.109
PCA	Graminoids	Time	1-7	1.15	0.319
	Shrubs	Time	1-7	0.83	0.393
FOG	Graminoids	Time	1-7	0.96	0.361
	Shrubs	Time	1-7	0.12	0.738

Fig. 4 Sum of standardized cover of plant functional types in 2001 and 2015 at the Target Region level. Horizontal bold line is the median, and the boxes are limited to the 1st and 3rd quartiles. Asterisks indicate significant changes between the two years (*: $p < 0.05$).

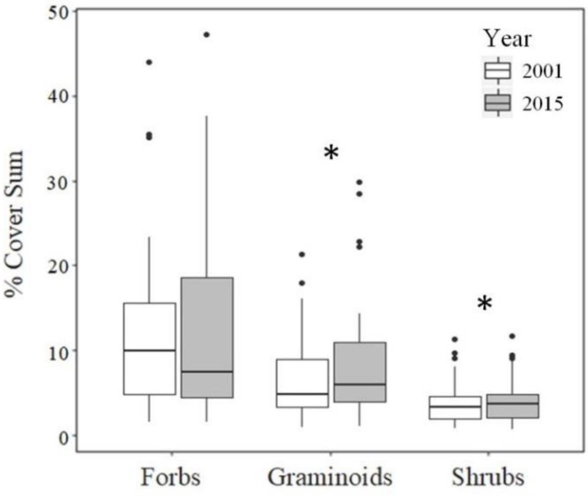
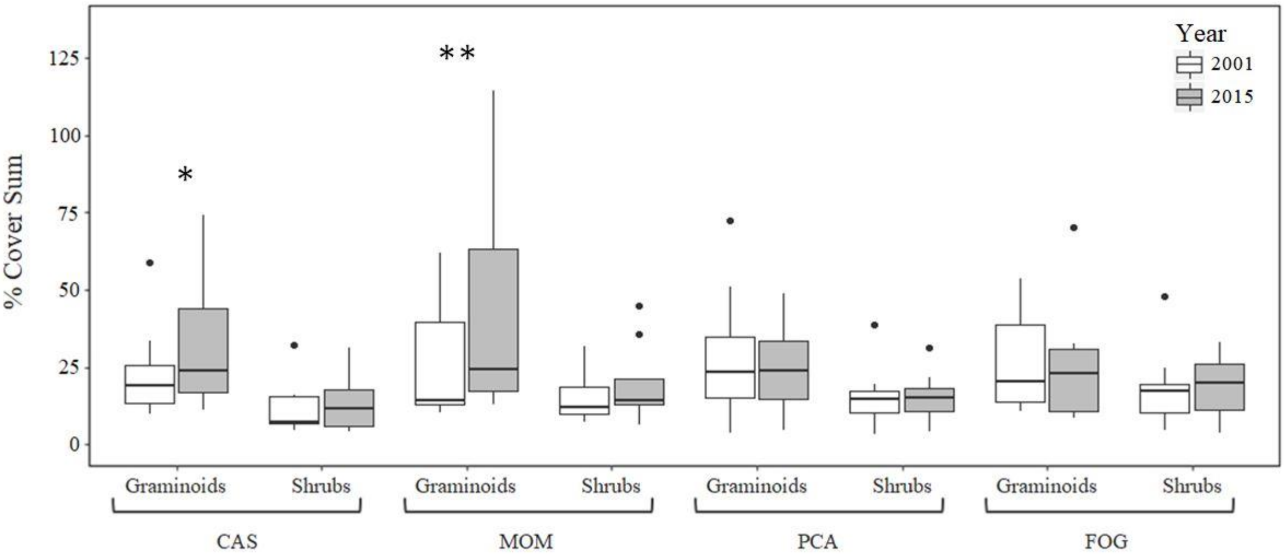


Fig. 5 Sum of standardized cover of plant functional types in 2001 and 2015 at the summit level. Horizontal bold line is the median, and the boxes are limited to the 1st and 3rd quartiles. Asterisks indicate significant changes between the two years (*: $p < 0.05$; **: $p < 0.01$).



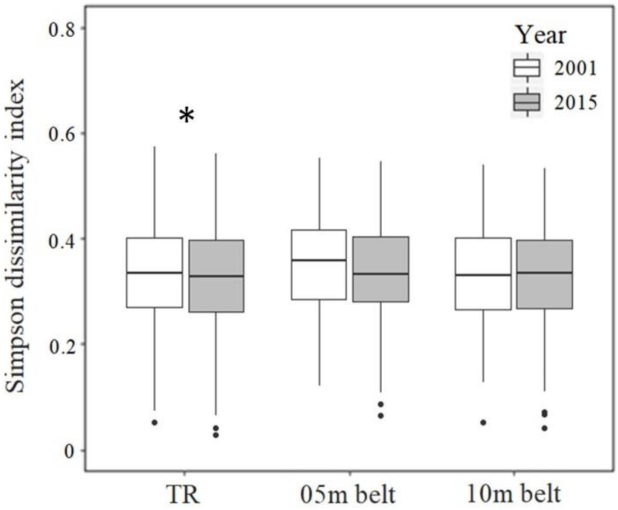
Assessment of β-diversity

The "multiple-site β_{SIM}" index values (indicating the turnover component of β-diversity) were considerably higher in comparison with the ones of the "multiple-site β_{SNE}" (indicating the nestedness component of β-diversity) across the years, with β_{SIM} index values always > 0.8 and β_{SNE} index values always < 0.05, showing that the β-diversity patterns in the studied area are mainly influenced by the species turnover. At the Target Region level, the "multiple-site β_{SIM}" index showed a decrease between 2001 and 2015, with a stronger loss of dissimilarity among SAS in the upper belts compared to the lower ones (Table 4). The "multiple-site β_{SIM}" values were higher in the upper belts compared to the lower ones both in 2001 and 2015, but a stronger decrease in dissimilarity was found in the upper ones. Similar to the "multiple-site β_{SIM}", expressed as an overall dissimilarity among all the 32 SAS, the "pairwise β_{sim}" index was lower in 2015 compared to 2001 at the Target Region level, whereas the changes at the belt levels were not significant (Fig. 6).

Table 4 β-diversity multiple-site Simpson dissimilarity index (β_{SIM} *sensu* Baselga 2010) at the Target Region (32 SAS included) and at upper and lower belt level (each including 16 SAS) in 2001 and 2015

Scale level	**β_{SIM} index**	
	2001	**2015**
Target Region	0.834	0.829
Upper belt	0.736	0.722
Lower belt	0.722	0.720

Fig. 6 Pair-wise Simpson dissimilarity index (β_{sim} *sensu* Baselga, 2010) at the Target Region level and at the upper and lower belt levels in 2001 and 2015. Horizontal bold line is the median, and the boxes are limited to the 1st and 3rd quartiles. Asterisks indicate significant changes between the two years (*: $p < 0.05$).



2.5 Discussion

The results of this study showed that, despite the relatively short time span of the investigations, significant changes occurred in the northern Apennines vegetation along elevation above tree-line. The integration of different biodiversity metrics has provided useful data for understanding the processes that have led to a general loss of diversity in these mountain summits. We have hence confirmed that a range of biodiversity metrics can give an accurate picture of change and allows assessment of biodiversity trends to support conservation and management decision making.

Species richness

The number of vascular species increased from 2001 to 2015 in the Target Region as a whole (Tables 1a, S3). When the data were split into summits and belts, a substantial increase was observed only on the two lower summits (PCA and FOG) and especially in their lower SAS. The asymmetry of the changes was due to the marked prevalence of colonization on loss events at these SAS. This result suggests the possibility that an increase in species richness may be supported by an upward migration. Species upward migration is consistent with expectations based on climate warming scenarios, and it was clearly detected by many recent observational studies and reviews from temperate and boreal mountains across Europe (Pauli et al. 2012; Grytnes et al. 2014; Steinbauer et al. 2018 among others). Taking into account that the four summits selected for the GLORIA Target Region are (and were) free from antropogenetic impacts (such as grazing or trampling), a possible role of climatic drivers for the upward migration can be hypothesized, as also suggested by the occurrence of several understory species of beech woodlands and their fringes (*Dactylorhiza fuchsii*, *Hieracium murorum*, *Luzula pedemontana* and *Silene nutans*) and of species of montane xerophytic grasslands (*Knautia purpurea* var. *calabrica* and *Asperula aristata* subsp. *oreophila*) and meadows (*Trollius europaeus*), in the 2015 survey, in both PCA and FOG.

The lack of significant variation in species richness between 2008 and 2015, along with the increment in species loss detected and the decrement of new species recorded in 2015 with respect to 2008, may indicate that not only the magnitude, but also the temperate-boreal direction of changes observed in the northern Apennine summits between 2001 and 2008 (see Pauli et al. 2012) were not confirmed in 2015. This suggests that a trend to species loss is possibly taking place since 2008 in the GLORIA summits of the northern Apennines. In the future, this could make the vegetation dynamics of the northern Apennine mountain summits more similar to the ones occurring in the Central Apennines, where a "Mediterranean" trend of species losses was already recorded between 2001 and 2008 on the higher summits (Pauli et al. 2012). This may lead to a northward shift of the border between temperate-boreal and Mediterranean summits across the Italian peninsula. Further support for this hypothesis is provided by the variation of species richness at the summit level. In the Target Region investigated, species richness showed no significant variation from 2001 to 2015 on the two higher summits and involved the loss of some of the most cold-adapted species occurring in the Target Region (*Arenaria moehringioides*, *Sagina glabra* and *Viola biflora*), as reported in the lost species list of our study sites (Tables S6, S7).

Starting from these two species lists, conservation actions such as seed collection (to be performed outside the Target Regions, following the international standards by ENSCONET 2009) for *ex situ* conservation or plant translocation for *in situ* conservation could be planned in the next future. Successful seed collection activities have already been carried out on the Alps by the Alpine Seed Conservation and Research Network (Müller et al. 2017) in which the Plant Germplasm Bank of the University of Pavia had an active role. A similar approach could be replicated in the Apennines and other mountain systems, taking into account the vegetation dynamics assessed within the GLORIA Target Regions, in order to collect seeds not only of already endangered species, but also of species that could be threatened in a long-term scenario (e.g. species with a constant decreasing abundance trend detected within a Target Region).

The role of global warming as a possible driver of this trend is supported by the climatic data documenting the combination of rising summer temperature and decreasing of summer precipitations in the northern Apennines (Antolini et al. 2017) and by a significant increase of summer temperature recorded at the Meteorological Observatory of Mt. Cimone, close to PCA summit (Fig. 1b). Moreover, Abeli et al. (2012a, b) and Orsenigo et al. (2015) already reported how warmer temperatures, heat waves, drought and reduced snow cover persistence can strongly affect both biomass production and reproduction of plant populations in the northern Apennines. However, some caution must be adopted when interpreting the variation of species richness trends over a time span of only seven years (between 2008 and 2015), also considering that the disappearance of species observed may either be the final stage of a population decline or the result of an unsuccessful species establishment, which are difficult to disentangle (Grytnes et al. 2014).

Assessment of α-diversity

Until about the beginning of this decade, it was predicted that the vegetation in the alpine life zone could resist long enough to climate change, showing a relative inertia in species composition, since slow growth rates, clonal abilities and longevity could delay the disappearance of species from habitats becoming climatically unsuitable (Theurillat and Guisan, 2001; Grabherr, 2003; Körner 2003; Dullinger et al. 2012). However, recent studies have showed that even closed alpine and subalpine communities may be considerably more dynamic than had previously thought, with changes in species richness and diversity over 25-50 years or shorter timescales (Ross et al. 2012; Cannone and Pignatti 2014; Carbognani et al. 2014; Matteodo et al. 2016 and others). When species richness and diversity were simultaneously analyzed, declines in diversity indices, alternatively combined with increase or decrease of species richness, were found and differently explained by several authors (Britton et al. 2009; Ross et al. 2012).

Diversity profiles provide a comprehensive and concise view of various facets of α-diversity of plant assemblages, and their variation over time reflects the balance among different ongoing processes driving the vegetation dynamics, such as upward migration, local extinction, competition and gap filling. Overall, greater vegetation diversity, in term of species richness, was found on the higher summits, where, however, the greatest loss of α-diversity is occurring. These diversity trends are consistent with a positive balance between species gains and losses in lower elevation summits, and a null or negative balance in higher elevation ones, affecting diversity profiles at low q values. In addition, the lowering of the profiles over time indicate an overall decrease in evenness among species, due to the combined effects of the appearance of new species (e.g. by upward migration) (Steinbauer et al. 2018; Wipf et al. 2013) and the expansion of abundant species (e.g. by gap filling) (Cannone and Pignatti 2014; Rumpf et al. 2018), with the latter process affecting diversity profiles at high q values, and more markedly in the higher summits.

Our results show the peculiarity that different patterns of species richness and diversity changes can occur within the same mountain range, even among summits with small differences in terms of elevation (of about 250 m or less). This can be explained by the fact that this gradient approximately embraces the whole alpine life zone of the northern Apennines, characterized by a high degree of local microhabitat diversity and heterogeneity in vegetation composition and structure within a relatively narrow surface (see Tomaselli 1994, Ferrari and Piccoli 1997; Tomaselli et al. 2000; Petraglia and Tomaselli 2007; Gennai et al. 2014).

Cover changes of plant functional types

We tried to understand the reason underlying the different patterns of α-diversity observed in the Target Region and in the four summits starting from the consideration that where α-diversity decreased (e.g. in term of diversity profile surface area), dominance regularly increased. Following this rationale, we observed that, among the functional types, only graminoid species experienced a significant increase of their total cover on the two higher summits, where dominance increased the most, while both graminoids and shrubs significantly increased at the Target Region level. Among graminoids, the endemic grass *Festuca riccerii* showed the highest increase in total cover in both CAS and MOM; other graminoids that increased on these summits were *Deschampsia flexuosa*, *Festuca rubra* subsp. *commutata* and *Juncus trifidus* (Fig. 7 in Appendix). A significant increase of graminoid species cover in upland heaths and grasslands was already reported by Ross et al. (2012) for the northwest Highlands of Scotland and by Cannone and Pignatti (2014) and Stanisci et al. (2016) for the Italian Alps and central Apennines. Differently, other authors demonstrated a high stability of species cover in siliceous grasslands from the northern Alps (Vittoz et al. 2009; Windmaißer and Reisch 2013; Matteodo et al. 2016). We must consider that the habitat conditions affecting the vegetation on the summits of our Target Region resemble those occurring on the windy ridges of the Alps, from where also Matteodo et al. (2016) reported significant changes in species composition, and of Scottish Highlands. On the other hand, Elmendorf et al. (2012) and Walker et al. (2006) suggested that graminoids could expand also in several Arctic, alpine and tundra sites as a consequence of experimental climate warming. Warmer temperatures can stimulate the mineralization of nitrogen in the soil (e.g. MacDonald et al. 1995; Rustad et al. 2001), increasing the nutrient availability for plant growth. Based on a 3-year experiment of nutrient addition in a summit heathland lying in the northern Apennines, Gerdol et al. (2000) proved that fertilization did not increase the aboveground biomass of ericaceous shrubs but, conversely, increase the one of graminoids. As suggested by Bobbink et al. (2010), due to increasing nitrogen concentration in the soil, vegetation changes have to be expected in natural environments, and as highlighted by Rumpf et al. (2018), thermophilic and nitrophilous species (as the graminoids experiencing abundance increments in our Target Region) are expected to become more abundant. With regard to shrub species, a generalized increment of their abundance has been detected in several arctic, and high latitude alpine tundra habitats as a consequence of climate warming (Myers-Smith et al. 2011; Myers-Smith et al. 2015). Similarly, we also observed a shrub species cover increment in our study site, at the Target Region Level. However, the thermophilous shrub *Juniperus communis* subsp. *alpina* is the only species which showed a sensible cover increment (see Fig. S1), while ericaceous shrub species cover remained overall stable. As such, a potential shrubification process of our area could be related to the cover increment of only one species so far, resulting thus less pivotal for the vegetation dynamics of the Target region than of other cold environments.

Our results seem to fit with the abovementioned findings, in particular when referring to the higher summits, where shrub cover was relatively stable, while graminoid species became more abundant (Fig. 4), also by colonizing open areas (average SAS vegetation cover increased over bare ground/rock cover by 1.8% in CAS and by 5.125% in MOM). At the two lower summits (PCA and FOG), instead, the diversity profile surface area decreased, while Dominance index increased only in FOG, where the abundance of *Juniperus communis* subsp. *alpina* increased the most (Fig. S1 in Appendix). PCA summit was the only one where both species richness and diversity increased, likely due to the particular feature of this summit, characterized by widespread open areas, with a very low vegetation cover, and no clear trend in term of plant functional type dynamics was observed.

Assessment of β-diversity

The higher absolute values of "multiple-site β_{SIM}" in comparison with the ones of "multiple-site β_{SNE}" indicate that in the northern Apennines summits the species turnover (i.e. replacement) has a prevailing importance over nestedness (i.e. species loss) in causing β-diversity patterns of vegetation. This result is likely due to the marked differences in vegetation composition among SAS with different aspect; as highlighted by Winkler et al. 2016, different aspect conditions can heavily influence species richness and species colonization, especially in temperate alpine summits, and thus could result as a key driver for summits β-biodiversity. The predominance of species turnover over nestedness suggests, once more, a "Mediterranean" pattern of vegetation diversity in the northern Apennines, consistent with the findings of Baselga (2010), indicating that β-diversity of longhorn beetle fauna in southern Europe is almost completely caused by species replacement only, whereas in northern Europe both turnover and nestedness contribute to β-diversity patterns.

Biotic homogenization as a consequence of upward migrated species arrival, has been recently documented in subalpine and alpine vegetation of several European mountain systems (Jurasinski and Kreyling 2007; Britton et al. 2009; Ross et al. 2012; Cannone and Pignatti 2014; Matteodo et al. 2016). The analysis of β-diversity trends between 2001 and 2015 showed that a process of homogenization is ongoing also within our Target Region, with a decrease of both multiple-site β_{SIM} and pairwise β_{sim} indices, indicating an increase of similarity of plant assemblages. The substantial asymmetry of changes resulting from the other biodiversity metrics analysis was confirmed also for the homogenization process. Dissimilarity loss is stronger in the upper belts, as they were less affected by the upward migration of new species. Nevertheless, the results obtained from the pairwise β_{sim} index calculation were not significant at the belt level. This indicates a weak homogenization signal, which is, however, more difficult to detect with fewer data, especially in the short time span of only fifteen years (Jurasinski and Kreyling 2007).

2.6 Concluding remarks

Our results confirmed the effectiveness of a multi-faceted approach for assessing plant diversity changes, capable of deepening the way and entity of the modification processes in the summits investigated. Overall, the diversity of the study site decreased over time, despite the increased species richness recorded in the period 2001-2015. In particular, there were evidences of a spatial asymmetry of both species richness and diversity variation along an elevational gradient: species enrichment was more effective on lower summits (and lower SAS belts) due to upward migration, whereas in higher summits species richness remained unvaried. Cover of graminoids and shrubs increased at the Target Region level, with a particularly marked graminoid species cover increment recorded on the higher summits, causing a reduction of the vegetation evenness and diversity. These findings, along with the species turnover and species loss recorded at the study site, suggest the presence of an ongoing process of homogenization and biodiversity loss. Moreover, as the study area is located at the southern border of the "temperate" mountains in Europe, the temporal trends in variation of species richness observed could be interpreted as an alarming signal, suggesting that a potential northward shift of the species richness dynamics observed in 2012 within the Mediterranean GLORIA Target Regions characterized by species loss (Pauli et al. 2012) could take place. Consequently, a transformation of the vegetation could be expected in the next decades also in the northern Apennines, and conservation activities, such as *ex/in situ* conservation actions are needed in the very next future, especially for plant species that are already declining in the studied mountain summits.

As a final consideration, we can say that the multi-faceted approach proposed in this study for the assessment of biodiversity over time could be effectively applied to the long-term vegetation data collected in the other GLORIA Target Regions. The diversity measures used in this case study are responsive to the vegetation changes recorded over time, despite the not so long period of monitoring considered. They can give valuable information about diversity dynamics of a site, detecting possible diversity loss and ongoing homogenization processes, which could be overlooked. The potential application of these methods within the GLORIA network (that include more than 120 sites), could thus result in a useful tool to highlight the areas most threatened by plant diversity loss in high elevation habitats all over the world.

Acknowledgements

The Grant of Excellence Departments, MIUR-Italy (ARTICOLO 1, COMMI 314-337 LEGGE 232/2016) is gratefully acknowledged for the support to one of the authors (Prof. Thomas Abeli). We also sincerely thank Anna Maria Cristina Antoniotti for her precious help during the fieldwork campaigns and two anonymous reviewers for critically reading the manuscript and for providing useful suggestions that helped to improve and clarify it.

2.7 References

- Abeli, T., Rossi, G., Gentili, R., Gandini, M., Mondoni, A., and Cristofanelli, P. (2012a). Effect of the extreme summer heat waves on isolated populations of two orophitic plants in the north Apennines (Italy). Nordic Journal of Botany, 30(1), 109-115.
- Abeli, T., Rossi, G., Gentili, R., Mondoni, A., and Cristofanelli, P. (2012b). Response of alpine plant flower production to temperature and snow cover fluctuation at the species range boundary. Plant Ecology, 213(1), 1-13.
- Alexander, J. M., Chalmandrier, L., Lenoir, J., Burgess, T. I., Essl, F., Haider, S., ... and Pauchard, A. (2018). Lags in the response of mountain plant communities to climate change. Global change biology, 24(2), 563-579.
- Antolini, G. (2017). Atlante Climatico dell'Emilia-Romagna 1961-2015. Casma Tipolito, Bologna, Italy.
- Atkinson, K. E. (2008). An introduction to numerical analysis. John Wiley and Sons.
- Baselga, A. (2010). Partitioning the turnover and nestedness components of beta diversity. Global ecology and biogeography, 19(1), 134-143.
- Baselga, A., and Orme, C. D. L. (2012). betapart: an R package for the study of beta diversity. Methods in Ecology and Evolution, 3(5), 808-812.
- Baselga, A. (2013). Multiple site dissimilarity quantifies compositional heterogeneity among several sites, while average pairwise dissimilarity may be misleading. Ecography, 36(2), 124-128.
- Barwell, L. J., Isaac, N. J., and Kunin, W. E. (2015). Measuring β-diversity with species abundance data. Journal of Animal Ecology, 84(4), 1112-1122.
- Bates, D., Mächler, M., Bolker, B., and Walker, S. (2014). Fitting linear mixed-effects models using lme4. arXiv preprint arXiv:1406.5823.
- Bellard, C., Bertelsmeier, C., Leadley, P., Thuiller, W., and Courchamp, F. (2012). Impacts of climate change on the future of biodiversity. Ecology letters, 15(4), 365-377.
- Bigelow, N. H., Brubaker, L. B., Edwards, M. E., Harrison, S. P., Prentice, I. C., Anderson, P. M., ... and Kaplan, J. O. (2003). Climate change and Arctic ecosystems: 1. Vegetation changes north of 55° N between the last glacial maximum, mid-Holocene, and present. Journal of Geophysical Research: Atmospheres, 108(D19).

- Britton, A. J., Beale, C. M., Towers, W., and Hewison, R. L. (2009). Biodiversity gains and losses: evidence for homogenisation of Scottish alpine vegetation. Biological conservation, 142(8), 1728-1739.
- Braun-Blanquet, J. (1964). Pflanzensoziologie: Grundzüge der Vegetationskunde Springer. New York, NY, US.
- Bobbink, R., Hicks, K., Galloway, J., Spranger, T., Alkemade, R., Ashmore, M., ... and Emmett, B. (2010). Global assessment of nitrogen deposition effects on terrestrial plant diversity: a synthesis. Ecological applications, 20(1), 30-59.
- Cannone, N., and Pignatti, S. (2014). Ecological responses of plant species and communities to climate warming: upward shift or range filling processes?. Climatic Change, 123(2), 201-214.
- Carbognani, M., Tomaselli, M., and Petraglia, A. (2014). Current vegetation changes in an alpine late snowbed community in the south-eastern Alps (N-Italy). Alpine botany, 124(2), 105-113.
- Carbognani, M., Bernareggi, G., Perucco, F., Tomaselli, M., and Petraglia, A. (2016). Micro-climatic controls and warming effects on flowering time in alpine snowbeds. Oecologia, 182(2), 573-585.
- Chao, A., Chiu, C. H., and Jost, L. (2014). Unifying species diversity, phylogenetic diversity, functional diversity, and related similarity and differentiation measures through Hill numbers. Annual review of ecology, evolution, and systematics, 45, 297-324.
- Champely, S. (2018). PairedData: Paired Data Analysis. R package version 1.1.1. https://CRAN.R-project.org/package=PairedData
- Chapin III, F. S., Bret-Harte, M. S., Hobbie, S. E., and Zhong, H. (1996). Plant functional types as predictors of transient responses of arctic vegetation to global change. Journal of vegetation Science, 7(3), 347-358.
- Colwell, R. K., Brehm, G., Cardelús, C. L., Gilman, A. C., and Longino, J. T. (2008). Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. science, 322(5899), 258-261.
- Dallan Nardi, L., and Nardi R. (1974). Schema stratigrafico e strutturale dell’Appennino settentrionale. Memorie della Accademia Lunigianese di Scienze 42 (1972):1–212.
- Di Battista, T., Fortuna, F., and Maturo, F. (2017). BioFTF: an R package for biodiversity assessment with the functional data analysis approach. Ecological indicators, 73, 726-732.

- Dullinger, S., Gattringer, A., Thuiller, W., Moser, D., Zimmermann, N. E., Guisan, A., ... and Caccianiga, M. (2012). Extinction debt of high-mountain plants under twenty-first-century climate change. Nature Climate Change, 2(8), 619-622.
- Elmendorf, S. C., Henry, G. H., Hollister, R. D., Björk, R. G., Bjorkman, A. D., Callaghan, T. V., ... and Fosaa, A. M. (2012). Global assessment of experimental climate warming on tundra vegetation: heterogeneity over space and time. Ecology letters, 15(2), 164-175.
- ENSCONET. (2009). ENSCONET seed collecting manual for wild species. http://www.plants2020.net/document/0183/
- Ernakovich, J. G., Hopping, K. A., Berdanier, A. B., Simpson, R. T., Kachergis, E. J., Steltzer, H., and Wallenstein, M. D. (2014). Predicted responses of arctic and alpine ecosystems to altered seasonality under climate change. Global Change Biology, 20(10), 3256-3269.
- Ferrari, C., and Piccoli, F. (1997). The ericaceous dwarf shrublands above the Northern Apennine timberline (Italy). Phytocoenologia, 53-76.
- Fox, J., Weisberg, S., Adler, D., Bates, D., Baud-Bovy, G., Ellison, S., ... and Heiberger, R. (2012). Package 'car'. Vienna: R Foundation for Statistical Computing. project.org/web/packages/car/car.pdf
- Fu, G., Shen, Z. X., Sun, W., Zhong, Z. M., Zhang, X. Z., and Zhou, Y. T. (2015). A meta-analysis of the effects of experimental warming on plant physiology and growth on the Tibetan Plateau. Journal of Plant Growth Regulation, 34(1), 57-65.
- Gennai, M., Foggi, B., Viciani, D., Carbognani, M., and Tomaselli, M. (2014). The Nardus-rich communities in the northern Apennines (N-Italy): a phytosociological, ecological and phytogeographical study. Phytocoenologia, 44(1-2), 55-85.
- Gerdol, R., Brancaleoni, L., Menghini, M., and Marchesini, R. (2000). Response of dwarf shrubs to neighbour removal and nutrient addition and their influence on community structure in a subalpine heath. Journal of Ecology, 88(2), 256-266.
- Grabherr, G., Gottfried, M., and Pauli, H. (1994). Climate effects on mountain plants. Nature, 369(6480), 448-448.
- Grabherr, G. (2003). Alpine vegetation dynamics and climate change-A synthesis of long-term studies and observations. In Alpine biodiversity in Europe (pp. 399-409). Springer, Berlin, Heidelberg.
- Grytnes, J. A., Kapfer, J., Jurasinski, G., Birks, H. H., Henriksen, H., Klanderud, K., ... and Birks, H. J. B. (2014). Identifying the driving factors behind observed elevational range shifts on European mountains. Global Ecology and Biogeography, 23(8), 876-884.

- Hammer, Ø., Harper, D. A., and Ryan, P. D. (2001). PAST: paleontological statistics software package for education and data analysis. Palaeontologia electronica, 4(1), 9.
- Hill, M. O. (1973). Diversity and evenness: a unifying notation and its consequences. Ecology, 54(2), 427-432.
- Holzinger, B., Hülber, K., Camenisch, M., and Grabherr, G. (2008). Changes in plant species richness over the last century in the eastern Swiss Alps: elevational gradient, bedrock effects and migration rates. Plant Ecology, 195(2), 179-196.
- IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland.
- Jurasinski, G., and Kreyling, J. (2007). Upward shift of alpine plants increases floristic similarity of mountain summits. Journal of Vegetation science, 18(5), 711-718.
- Kirschbaum, M. U. (1995). The temperature dependence of soil organic matter decomposition, and the effect of global warming on soil organic C storage. Soil Biology and biochemistry, 27(6), 753-760.
- Koleff, P., Gaston, K. J., and Lennon, J. J. (2003). Measuring beta diversity for presence–absence data. Journal of Animal Ecology, 72(3), 367-382.
- Körner, C. (1995). Towards a better experimental basis for upscaling plant responses to elevated CO2 and climate warming. Plant, Cell and Environment, 18(10), 1101-1110.
- Körner, C. (2003). Alpine plant life: functional plant ecology of high mountain ecosystems; with 47 tables. Springer Science and Business Media.
- Körner, C., Jetz, W., Paulsen, J., Payne, D., Rudmann-Maurer, K., and Spehn, E. M. (2017). A global inventory of mountains for bio-geographical applications. Alpine Botany, 127(1), 1-15.
- Lenoir, J., Gégout, J. C., Marquet, P. A., De Ruffray, P., and Brisse, H. (2008). A significant upward shift in plant species optimum elevation during the 20th century. science, 320(5884), 1768-1771.
- MacDonald, N. W., Zak, D. R., and Pregitzer, K. S. (1995). Temperature effects on kinetics of microbial respiration and net nitrogen and sulfur mineralization. Soil Science Society of America Journal, 59(1), 233-240.
- Matteodo, M., Ammann, K., Verrecchia, E. P., and Vittoz, P. (2016). Snowbeds are more affected than other subalpine–alpine plant communities by climate change in the Swiss Alps. Ecology and evolution, 6(19), 6969-6982.

- Myers-Smith, I. H., Elmendorf, S. C., Beck, P. S., Wilmking, M., Hallinger, M., Blok, D., ... and Speed, J. D. (2015). Climate sensitivity of shrub growth across the tundra biome. Nature Climate Change, 5(9), 887.
- Myers-Smith, I. H., Forbes, B. C., Wilmking, M., Hallinger, M., Lantz, T., Blok, D., ... and Boudreau, S. (2011). Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. Environmental Research Letters, 6(4), 045509.
- Mountain Research Initiative EDW Working Group (2015). Elevation-dependent warming in mountain regions of the world. Nature Climate Change, 5(5), 424-430.
- Müller, J. V., Berg, C., Détraz-Méroz, J., Erschbamer, B., Fort, N., Lambelet-Haueter, C., ... and Porro, F. (2017). The Alpine Seed Conservation and Research Network-a new initiative to conserve valuable plant species in the European Alps. Journal of Mountain Science, 14(4), 806-810.
- Orsenigo, S., Mondoni, A., Rossi, G., and Abeli, T. (2014). Some like it hot and some like it cold, but not too much: plant responses to climate extremes. Plant Ecology, 215(7), 677-688.
- Orsenigo, S., Abeli, T., Rossi, G., Bonasoni, P., Pasquaretta, C., Gandini, M., and Mondoni, A. (2015). Effects of autumn and spring heat waves on seed germination of high mountain plants. PloS one, 10(7), e0133626. doi:10.1371/journal.pone.0133626
- Parmesan, C. (2006). Ecological and evolutionary responses to recent climate change. Annu. Rev. Ecol. Evol. Syst., 37, 637-669.
- Parolo, G., and Rossi, G. (2008). Upward migration of vascular plants following a climate warming trend in the Alps. Basic and Applied Ecology, 9(2), 100-107.
- Patil, G. P., and Taillie C. (1979). An overview of diversity. Ecological diversity in theory and practice. Fairland (Maryland), International Cooperative Publishing House, pp 3-27.
- Patil, G. P., and Taillie, C. (1982). Diversity as a concept and its measurement. Journal of the American statistical Association, 77(379), 548-561.
- Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Alonso, J. L. B., ... and Ghosn, D. (2012). Recent plant diversity changes on Europe’s mountain summits. Science, 336(6079), 353-355.
- Pauli, H., Gottfried, M., and Grabherr, G. (2014). Effects of climate change on the alpine and nival vegetation of the Alps. Journal of mountain ecology, 7.
- Pauli, H., Gottfried, M., Lamprecht, A., Niessner, S., Rumpf, S., Winkler, M., ... and Grabherr, G. (2015). The GLORIA field manual—standard Multi-Summit approach, supplementary methods and extra approaches. Vienna: GLORIA-Coordination, Austrian Academy of Sciences and University of Natural Resources and Life Sciences.

- Petraglia, A., and Tomaselli, M. (2007). Phytosociological study of the snowbed vegetation in the Northern Apennines (Northern Italy). Phytocoenologia, 37(1), 67-98.
- Pielou, E. C. (1975). Ecological diversity. John Wiley et sons.
- R Core Team (2017). R: A language and environment for statistical com-puting. R Foundation for Statistical Computing, Vienna, Austria.
- Rixen, C., Wipf, S., Frei, E., and Stöckli, V. (2014). Faster, higher, more? Past, present and future dynamics of alpine and arctic flora under climate change. Alpine Botany, 124(2), 77-79.
- Rogora, M., Mosello, R., Arisci, S., Brizzio, M. C., Barbieri, A., Balestrini, R., ... and Kalina, M. (2006). An overview of atmospheric deposition chemistry over the Alps: present status and long-term trends. Hydrobiologia, 562(1), 17-40.
- Ross, L. C., Woodin, S. J., Hester, A. J., Thompson, D. B., and Birks, H. J. B. (2012). Biotic homogenization of upland vegetation: patterns and drivers at multiple spatial scales over five decades. Journal of vegetation science, 23(4), 755-770.
- Rumpf, S. B., Hülber, K., Klonner, G., Moser, D., Schütz, M., Wessely, J., ... and Dullinger, S. (2018). Range dynamics of mountain plants decrease with elevation. Proceedings of the National Academy of Sciences, 115(8), 1848-1853.
- Rumpf, S. B., Hülber, K., Zimmermann, N. E., and Dullinger, S. (2019). Elevational rear edges shifted at least as much as leading edges over the last century. Global Ecology and Biogeography, 28(4), 533-543.
- Rustad, L. E. J. L., Campbell, J., Marion, G., Norby, R., Mitchell, M., Hartley, A., ... and Gurevitch, J. (2001). A meta-analysis of the response of soil respiration, net nitrogen mineralization, and aboveground plant growth to experimental ecosystem warming. Oecologia, 126(4), 543-562.
- Sala, O. E., Chapin, F. S., Armesto, J. J., Berlow, E., Bloomfield, J., Dirzo, R., ... and Leemans, R. (2000). Global biodiversity scenarios for the year 2100. science, 287(5459), 1770-1774.
- Shannon, C. E. (1948). A mathematical theory of communication. Bell system technical journal, 27(3), 379-423.
- Sheldon, A. L. (1969). Equitability indices: dependence on the species count. Ecology, 50(3), 466-467.
- Simpson, E. H. (1949). Measurement of diversity. nature, 163(4148), 688-688.

- Stanisci, A., Pelino, G., and Blasi, C. (2005). Vascular plant diversity and climate change in the alpine belt of the central Apennines (Italy). Biodiversity and Conservation, 14(6), 1301-1318.
- Stanisci, A., Frate, L., Morra Di Cella, U., Pelino, G., Petey, M., Siniscalco, C., and Carranza, M. L. (2016). Short-term signals of climate change in Italian summit vegetation: observations at two GLORIA sites. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology, 150(2), 227-235.
- Steinbauer, M. J., Grytnes, J. A., Jurasinski, G., Kulonen, A., Lenoir, J., Pauli, H., ... and Bjorkman, A. D. (2018). Accelerated increase in plant species richness on mountain summits is linked to warming. Nature, 556(7700), 231-234.
- Theurillat, J. P., and Guisan, A. (2001). Potential impact of climate change on vegetation in the European Alps: a review. Climatic change, 50(1-2), 77-109.
- Thomas, H. J., Myers-Smith, I. H., Bjorkman, A. D., Elmendorf, S. C., Blok, D., Cornelissen, J. H., ... and Rixen, C. (2019). Traditional plant functional groups explain variation in economic but not size-related traits across the tundra biome. Global ecology and biogeography, 28(2), 78-95.
- Thompson, D. B. A., MacDonald, A. J., Marsden, J. H., and Galbraith, C. A. (1995). Upland heather moorland in Great Britain: a review of international importance, vegetation change and some objectives for nature conservation. Biological Conservation, 71(2), 163-178.
- Tomaselli, M. (1994). The vegetation of summit rock faces, talus slopes and grasslands in the northern Apennines (N Italy). Fitosociologia, 26, 35-50.
- Tomaselli, M., and Rossi, G. (1994). Phytosociology and ecology of Caricion curvulae vegetation in the northern Apennines (N Italy). Fitosociologia, 26, 51-62.
- Tomaselli, M., Rossi, G., and Dowgiallo, G. (2000). Phytosociology and ecology of the Festuca puccinellii-grasslands in the northern Apennines (N-Italy). Botanica Helvetica, 110(2), 125-149.
- Tóthmérész, B. (1998). On the characterization of scale-dependent diversity. Abstracta Botanica, 149-156.
- Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., and Webb, D. A. (1964). 1980: Flora europaea. Cambridge: Univ. Press, 1(1964), 428.
- Van der Maarel, E. (1979). Transformation of cover-abundance values in phytosociology and its effects on community similarity. Vegetatio, 39(2), 97-114.

- Vittoz, P., Randin, C., Dutoit, A., Bonnet, F., and Hegg, O. (2009). Low impact of climate change on subalpine grasslands in the Swiss Northern Alps. Global Change Biology, 15(1), 209-220.
- Vittoz, P., Bayfield, N., Brooker, R., Elston, D. A., Duff, E. I., Theurillat, J. P., and Guisan, A. (2010a). Reproducibility of species lists, visual cover estimates and frequency methods for recording high-mountain vegetation. Journal of Vegetation Science, 21(6), 1035-1047.
- Vittoz, P., Camenisch, M., Mayor, R., Miserere, L., Vust, M., and Theurillat, J. P. (2010b). Subalpine-nival gradient of species richness for vascular plants, bryophytes and lichens in the Swiss Inner Alps. Botanica Helvetica, 120(2), 139-149.
- Walker, M. D., Wahren, C. H., Hollister, R. D., Henry, G. H., Ahlquist, L. E., Alatalo, J. M., ... and Epstein, H. E. (2006). Plant community responses to experimental warming across the tundra biome. Proceedings of the National Academy of Sciences, 103(5), 1342-1346.
- Walther, G. R., Beißner, S., and Burga, C. A. (2005). Trends in the upward shift of alpine plants. Journal of Vegetation Science, 16(5), 541-548.
- Windmaißer, T., and Reisch, C. (2013). Long-term study of an alpine grassland: local constancy in times of global change. Alpine botany, 123(1), 1-6.
- Winkler, M., Lamprecht, A., Steinbauer, K., Hülber, K., Theurillat, J. P., Breiner, F., ... and Vittoz, P. (2016). The rich sides of mountain summits–a pan-European view on aspect preferences of alpine plants. Journal of Biogeography, 43(11), 2261-2273.
- Wipf, S., Stöckli, V., Herz, K., and Rixen, C. (2013). The oldest monitoring site of the Alps revisited: accelerated increase in plant species richness on Piz Linard summit since 1835. Plant Ecology and Diversity, 6(3-4), 447-455.
- Wullschleger, S. D., Epstein, H. E., Box, E. O., Euskirchen, E. S., Goswami, S., Iversen, C. M., ... and Xu, X. (2014). Plant functional types in Earth system models: past experiences and future directions for application of dynamic vegetation models in high-latitude ecosystems. Annals of botany, 114(1), 1-16.

Appendix

Table S1 Elevation, geographic coordinates and climatic features (1970-2000) and vegetation belt of the four selected summits. CAS: Mt. Casarola; MOM: Alpe di Mommio; PCA: Cima di Pian Cavallaro; FOG: Cima di Foce a Giovo. Long-term climatic features of the four summits were extracted from WorldClim 2 (reference period: 1970-2000; Fick and Hijmans 2017) at 30 seconds spatial resolution.

	CAS	**MOM**	**PCA**	**FOG**
Elevation (m a.s.l.)	1978	1855	1815	1722
Latitude (N)	44°19’53”	44°16’32”	44°12’09”	44°07’07”
Longitude (E)	10°12’37”	10°14’40”	10°41’33”	10°36’43”
Mean annual temperature (°C)	4.8	5.7	5.1	5.1
Mean annual precipitation (mm)	1055	1269	992	1065
Vegetation belt	Lower alpine	Higher subalpine	Lower subalpine	Treeline

Table S2 Gloria abundances classes codes and descriptors (Pauli et al. 2015), along with the converted cover approximation proposed following Braun-Blanquet.

GLORIA abundance codes	**GLORIA abundance classes descriptor**	**Converted % Cover approximation**	**Corresponding Braun-Blanquet scale codes**
r! – very rare	Few individuals within the SAS	0.1%	+
r – rare	Some individuals at several locations that can hardly be overlooked in a careful observation	5%	1
s – scattered	Widespread within the SAS. Can’t be overlooked but its presence could be not obvious at first glance	17.5%	2
c – common	Occurs frequently and is widespread within the section (cover < 50%)	37.5%	3
d - dominant	Very abundant, making up a high portion of the phytomass (cover > 50%)	75%	4-5

Table S3 Dataset composition and relative number of vascular species recorded in the three years of monitoring in the a) Target Region, b) summit and c) belt level. “SAS” indicates the number of summit area section included for each level, whereas 05m and 10m indicate the upper and lower belt of each summit, respectively.

Scale level	SAS	Number of species			Difference
		2001	2008	2015	2001−2015
a) Target Region	32	154	164	159	+5
b) summit					
CAS	8	107	104	107	0
MOM	8	96	98	90	-6
PCA	8	92	95	98	+6
FOG	8	77	85	83	+6
c) belt					
CAS 05m	4	89	88	93	+4
CAS 10m	4	102	99	98	-4
MOM 05m	4	83	81	77	-6
MOM 10m	4	81	86	79	-2
PCA 05m	4	87	87	88	+1
PCA 10m	4	80	90	94	+14
FOG 05m	4	65	69	67	+2
FOG 10m	4	69	78	80	+11

Table S4 Area (expressed in m^2) of all the 8 SAS of each summit (two per aspect) included in the Target Region IT-NAP. Upper and lower SAS are indicated by “5” and “10” respectively

Summit	East 5	East 10	North 5	North 10	South 5	South 10	West 5	West 10
CAS	155.36	392.09	229.1	567.71	171.04	264.38	214.73	809.53
MOM	139.5	155.52	131.15	441.31	169.07	212.29	534.05	597.93
PCA	304.55	725.26	53.75	133.06	225.23	465.38	398	394.24
FOG	72.63	504.98	109.15	198.07	84.00	215.37	290.12	254.48

Table S5 List of the species removed from the database.

Species
Astragalus purpureus Lam.
Bellardiochloa violacea (Bellardi) Chiov.
Carex pilulifera L.
Colchicum alpinum DC.
Gymnadenia conopsea (L.) R.Br.
Helianthemum nummularium (L.) Miller subsp. *nummularium*
Koeleria macrantha (Ledeb.) Schult.
Leucanthemum adustum (W.D.J. Koch) Gremli
Leucanthemum atratum (Jacq.) DC. subsp. *ceratophylloides* (All.) Horvatic
Pedicularis cenisia Gaudin
Polygonum viviparum L.
Scorzonera rosea Waldst. and Kit.
Sorbus aria (L.) Crantz
Unidentified *Hieracium* species

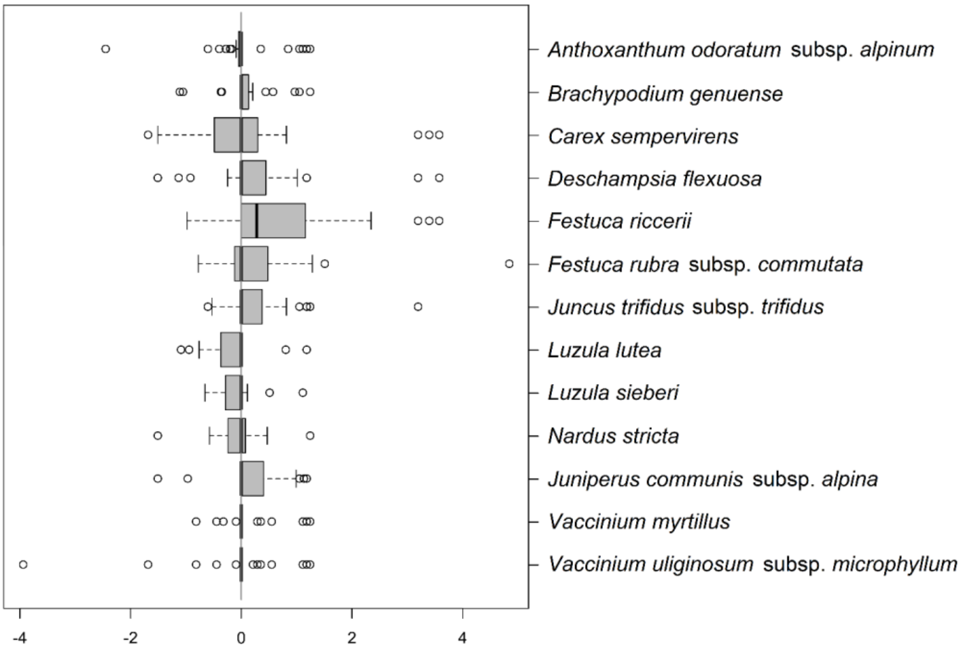
Table S6 List of the species disappeared from the Target Region.

Species
Crocus vernus (L.) Hill *subsp. vernus*
Deschampsia cespitosa (L.) P. Beauv. subsp. *cespitosa*
Maianthemum bifolium (L.) F.W. Schmidt
Rumex nebroides Campd.
Sagina glabra (Willd.) Fenzl
Taraxacum officinale Weber agg.
Cuscuta epithymum (L.) L. subsp. *epithymum*
Anemone nemorosa L.
Lilium bulbiferum L. subsp. *bulbiferum* var. *croceum* (Chaix) Pers.

Table S7 List of the species disappeared from at least one summit within the Target Region

Species
Alchemilla transiens (Buser) Buser
Allium senescens subsp. *montanum* (F.W. Schmidt) Holub
Arenaria moehringioides Murr
Asplenium trichomanes-ramosum L.
Aster alpinus L.
Biscutella laevigata L. subsp. *Laevigata*
Carduus carlinifolius Lam.
Galium anisophyllon Vill.
Galium x *centroniae* Cariot
Geranium sylvaticum L. subsp. *sylvaticum*
Hieracium lactucella Wallr.
Hieracium pilosella L.
Huperzia selago (L.) Bernh. ex Schrank and Mart. subsp. *selago*
Linum alpinum Jacq. subsp. *gracilius* (Bertol.) Pignatti
Myosotis alpestris F.W. Schmidt
Phyteuma orbiculare L.
Scabiosa lucida Vill.
Sedum monregalense Balb.
Viola biflora L.

Fig. 7 Difference in standardized cover of the most abundant graminoids and shrubs in the summit studied.



3. Comparative seed germination of alpine plants in the context of climate change

Abstract

In the context of climate warming seeds are thought to play an important role, being the main mean veicle for plant migration, establishment and persistence. Seed germination and seedling establishment are the most sensitive to variation in climate conditions and represent the major bottleneck in species recruitment. In alpine plants, seedling recruitment is under strong environmental control, suggesting that climate change will inevitably affect recruitment success. Here, I present the first attempt to evaluate the role of seed germination as a driver of species response to climate warming. I hypothesized that species showing a trend of increment or that are stable in nature germinate better at warmer temperatures, have 2) a wider suitable temperature range for germination and 3) are less dormant than species showing a decrement in term of abundance.

Seeds of 75 alpine species from five different study sites were collected between 2017 and 2018. The species were chosen and divided into five groups depending on whether their population trend observed along 15 years of monitoring (project GLORIA, http://www.gloria.ac.at/?a=2) was a) increased, b) decreased or c) stable; I also included d) new species and e) species that disappeared. Seed germination was tested in the laboratory at six constant temperatures (T, from 5° to 30°C with 5°C increment), with and without a cold stratification treatment (CS). Germination data were analyzed by the means of GLM. As expected, species increasing in nature consistently showed the highest final germination percentage and the widest range of suitable temperature. Contrary to my hypothesis, these species germinated better at cold T (5°C), after CS. Species from the other groups were characterized by an overall lower seed germination percentage, that increased after CS, but mostly at high T (20°C, 25°C).

Results indicate that species with high germination percentage at low temperatures may be favored in present and in a future warmer climate, by eliciting seedling emergence early in spring right after snow melt, when water is available and the hazards of drought is low. At the contrary, a delayed germination, synchronized with higher temperature may elicits seedling emergence in periods of the year becoming less suitable with climate warming.

Introduction

Studying plant functional traits is a powerful approach to summarize and simplify the structure or function of plant communities (McGill et al. 2006). As functional traits can be measured, and have implications for species fitness, they can be used to predict the performance of species, composition of communities and how they shift over time and space (Larson and Funk 2016). Additionally, the convergence of functional traits in species occupying similar environments can be considered a shared adaptation to environmental filtering (Keddy 1992). Identifying the most relevant plant functional traits for use in predictive frameworks is crucial to studies on community structure and composition (Funk et al. 2016), responses to climate warming (Orru et al. 2012; Valladares et al. 2014) and biotic or abiotic disturbance. In these contexts, incorporating regeneration traits has long been recognized as a particularly important goal in ecology (Weiher et al. 1999; Lavorel and Garnier 2002), for example, to understand plant community properties including stability and change (Donohue et al. 2010). However, except seed mass, there have been few attempts to integrate regeneration traits into community assembly models (Larson and Funk 2016). Nevertheless, for a species to be present in a plant community, firstly seeds must be dispersed or present, and secondly, appropriate environmental conditions for germination, plant growth, survival and persistence must be met (Donohue et al. 2010, Poschlod et al. 2013).

Regeneration niche is mainly limited by physical and chemical requirements for species' survival and reproduction including all processes encompassed in viable seed production, seed dispersal, seed germination and seedling survival (Grubb 1977, Saatkamp et al. 2019). Germination traits (in particular) are gaining more attention (Orru et al. 2012; Fernández-Pascual et al. 2015; Dürr et al. 2015; Ordoñez-Salanueva et al. 2015; Seal et al. 2017) and are now integrated in plant community ecology (Jiménez-Alfaro et al. 2016; Poschlod et al. 2013, Larson and Funk 2016). Additionally, metrics which capture the width of the germination niche (i.e. the range of conditions in which a population may germinate) could have explanatory power (Donohue et al. 2010). For example, using just a few physiological germination traits (e.g. thermal time, optimal temperature and base water potential for germination) along with precipitation and moisture data, Huang et al. (2016) were able to explain yearly variation in germination patterns across 13 desert annual species over a 25-year period. Consequently, a study highlighting germination and other regeneration traits-variation within communities may shed light on their functional role in driving species responses to climate warming.

A large body of evidence supports the existence of rapid climate change, the main effect of which is the rise in mean global temperatures (IPCC 2014). This is particularly evident in high-elevation biomes, making these ecosystems, inhabited by species highly specialized to low temperatures, among the environments most threatened by the predicted climate change (Diaz et al. 1997; Nogués-Bravo et al. 2007). As a consequence, in the last decades, an increasing number of studies have addressed the effects of climate warming on different life-history traits and functions of alpine ecosystems, documenting changes in phenology, physiology, distribution (as upward shift) of several alpine species (see e.g. Grabherr et al. 1995; Klanderud and Birks 2003; Walther et al. 2005; Lenoir et al. 2008; Parolo and Rossi 2008; Gottfried et al. 2012; Pauli et al. 2012). Importantly, long-term monitoring studies of alpine flora have already highlighted changes in species composition on several mountain zones in Europe, as a consequence of global warming (Pauli et al. 2012; Gottfried et al. 2012). Additionally, these studies often provide an evaluable knowledge base to address conservation actions and further researches, for example, founding out which species are currently more threatened, or investigating which plant traits (if any) makes species more sensitive to warming.

In the context of climate warming seeds are thought to play an important role, being the main mean of transport for plant migration (Parolo and Rossi 2008), regeneration (Walck et al. 2011) and persistence (Schwienbacher et al. 2010). The impact of climate change on regeneration from seeds is therefore of growing interest, especially in arctic (e.g. Shevtsova et al. 2009; Milbau et al. 2009) and in alpine (Mondoni et al. 2012, 2015; Hoyle et al. 2013, Bernareggi et al. 2015, 2016) environments. Indeed, among plant reproductive phases, seed germination and seedling establishment are probably the most sensitive to variation in climate conditions (Walck et al., 2011) and represent the major bottleneck to species recruitment (Lloret et al. 2004, Dalgleish et al. 2010). The strong correlation between climate and plant regeneration from seeds has resulted in the evolution of specific germination requirements across many species (Fenner et al. 2005), which play a key role in plant distribution and vegetation dynamics (Silvertown and Charlesworth 2001; Neilson et al. 2005; McGill et al. 2006; Pearson 2006).

In alpine plants, seed germination and seedling recruitment are under strong environmental control, being affected largely by snow cover period length, 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. In fact, seed germination of alpine/arctic species has been shown to vary considerably in response to climate warming, increasing, reducing or shifting in time (e.g. Milbau et al. 2009; Shevtsova et al. 2009; Mondoni et al. 2012; Hoyle et al. 2013; Briceño et al. 2015; Mondoni et al. 2015), thereby showing contrasting results. Despite this, little is known about the role of regeneration from seeds as key-plant functional traits driving changes of species presence and/or abundance due to climate warming, in part because of the technical difficulty involved in measuring these traits. However, understanding dormancy cues and germination requirements of species showing a decline due to climate warming is essential to predict population response and model trajectory.

Here, I present a first attempt to evaluate the role of germination ecology as driver of plant species response to climate warming. In particular, the aim of this study was to investigate whether species currently showing changes in their presence and abundance due to climate warming are characterized by different specific germination response to Temperature. To test this hypothesis, the long-term data series collected at the mountain peaks of the European project GLORIA (http://www.gloria.ac.at/europe/) represent important knowledge base, both in term of species and temperature changes occurred during the last decades. Among the active GLORIA sites (Target Regions) this study focuses on five areas located in the Central and Southern Alps, Northern and Central Apennines and southern Spain. Specifically I hypothesized that species currently showing persistence or an increase under a natural warming trend 1) have overall higher germination values, 2) show a wider window of suitable temperature for germination and 3) are characterized by less constrained environmental cues to break potential seed dormancy.

Materials and Methods

Study area and species selection

Study sites and target species have been chosen relying on the vegetation and climatic data collected all over the European alpine regions by the international project GLORIA - Global Observation Research Initiative in Alpine Environment (http://www.gloria.ac.at). GLORIA aims to establish and maintain a worldwide long-term observation network in alpine environments. To this end, several Target Region (TR hereafter), managed by research institutions and universities, are under monitoring since 2001. In each TR (4 summits with different position along an altitudinal gradient) a complete list of plant species including their frequency and abundance is carried out at five/eight-years intervals (depending on the monitoring site). Additionally, soil temperature is being monitored using loggers on each summit.

Among all European GLORIA TR, the following 5 sites have been chosen for the selection of the target species to include in this study: 1) Es-Sne, Sierra Nevada (Es), 2) It-Nap, Northern Apennines (It), 3) It-Maj, Central Apennines (It), 4) It-Ado, west Dolomites (It), 5) It-Tex, north Dolomites (It) (Fig. 1). These TR have been chosen because they are among the oldest in the network, thereby having the longest series of data available (i.e. > 15 years of monitoring activities, with at least 3 vegetation survey completed).

Fig. S1 Target Region map. 1 = Es-Sne; 2 = It-Nap; 3 = It-Maj; 4 = It-Ado; 5 = It-Tex.

Most importantly, these sites were chosen also to represent the two different main trends in species richness changes observed in the "Boreal/Temperate" and "Mediterranean" TR, as discussed in Pauli et al., (2012). For instance, It-Ado, It-Tex and It-Nap ("Boreal/Temperate" TR), and their summits were characterized by species richness increments over the first 8 years of monitoring activities, while species richness in It-Maj and Es-Sne ("Mediterranean" TR) remained stable or decreased.

According with the changes in species presence and abundance recorded in each site (by the project GLORIA), the target species have been selected considering the presence of a clear trend of increase, decrease, or stability through time. Plant species occurring on each TR were ranked taking into account both cover percentage and frequency data (24 plots for each TR, see GLORIA manual for details, Pauli et al. 2015), from those that show the highest positive increment to those showing the highest decrements, prioritizing abundance values. Subsequently, species have been be clustered in the following five categories (Population Trend Group, PTG hereafter):

1) [UP] species that showed an increase of abundance;
2) [DOWN] species that showed a decrease of abundance;
3) [STABLE] species that showed no change of frequency;

4) [NEW] new species for the TR, not found during the first survey;

5) [LOST] species that disappeared locally within the TR after the first survey.

An initial list of 178 species of interest from the five TR was realized. For each TR were selected at least 5 species per PTG, when possible (e.g. new and disappeared species were often <5 within each TR). In particular, each TR species list included: EsSne (30 sp.), ItNap (42 sp.), ItMaj (24 sp.), ItAdo (43 sp.), ItTex (39).

Table 1 Codes, Geographic location, TR site institution manager and altitudinal range of the 4 summits included in the five GLORIA TR chosen as study site.

TR Code	Region	Institution	Altitudinal range
It-Nap	Italy, Emilia-Romagna	University of Pavia, University of Parma	1722 - 1978 m a.s.l.
It-Maj	Italy, Abruzzo	University of L'Aquila	2405 - 2730 m a.s.l.
It-Ado	Italy, Trentino-Alto Adige	University of Innsbruck	2199 - 2893 m a.s.l.
It-Tex	Italy, Trentino-Alto Adige	University of Innsbruck	2180 - 3287 m a.s.l.
Es-Sne	Spain, Andalucía	University of Granada	2778 – 3327 m a.s.l.

Seed collection

Target species seeds were collected for subsequent germination experiments (Appendix S1) with two seed collecting campaigns. In 2017, 18 species out of 30 initial target species were collected in EsSne, 21 in ItNap, 14 in ItMaj and 6 in ItAdo. On average, more than 3000 seeds were collected for each species. In 2018, 8 more species were collected in itAdo, and 15 in ItTex. Because of issues related to seed availability (e.g. species producing not enough seeds) and laboratories constrains (e.g. max number of test to be set up concurrently, the total number of species tested was 75, with no less than 6 species per category (see Table S1). Seed collection of the target species were carried out following guidelines developed in the European projects ENSCONET (2009) and Way et al., (2003). Seed collection was carried out simultaneously during the species natural seed dispersal period; I sampled seeds from at least 25 different individuals accounting for less than the 20% of each population total seed production for that year. Seed collection activities have been performed in the surrounding areas of each GLORIA TR, outside the fixed plot.

Germination tests

All germination experiments were carried out by sowing 3 replicates of 25 seeds per treatment (with the exception of 8 species, see Table S1, for which only 15 seeds per replicates were sown due to seed shortage), on 1 % distilled water-agar held in 50-mm-diameter Petri dishes. Experiments were conducted in temperature and light-controlled incubators (LMS 250A, LMS Ltd, Sevenoaks, UK) at 12-h daily photoperiod (PAR 40 – 50 µmol m-2 s-1).

Petri dishes with fresh seeds were incubated under different daily constant temperatures ranging from 0 to 30 °C, with increments of 5 °C between each treatment. A second block of tests were exposed to a cold-wet stratification treatment at dark condition for four months and half (i.e. 130 days), which is the average period of snow cover length detected within the GLORIA TR chosen here. At the end of the stratification treatment, seeds were moved to the same incubation temperatures described above. The experiments started in October 2017 and October 2018, after each of the two seed collecting campaigns (see above). All experiments were terminated in July 2019. Germination plates were checked for radicle emergence (>2 mm) every 8 hours for the first week of tests and, subsequently at daily or weekly interval for 4 months, depending on the germination rate. At the end of the experiments, non-germinated seeds were cut-tested and the seeds empty or containing parasites (e.g. insect larvae) were counted as unviable. Then, final germination percentage (FGP) was calculated excluding non-viable seeds. Dormancy types and classes of each species were defined following the Baskin and Baskin (2014), Silveira's diagram (Silveira 2013) and the germination response obtained with the laboratory tests performed. Embryo morphology data were collected from the literature (Martin 1946). Additional information about the classification criteria used are reported in Table S2 (from Chapter 3). Target species dormancy classes are reported in Table S1.

Statistical analysis

All statistical analysis and plotting were performed by the mean of the R environment for statistical computing with graphics R studio version 3.6.1 (R Core Team 2019), and the software PAST (Hammer et al. 2015).

To investigate the effects of treatments on seed germination, generalized linear models (GLM) with binomial distribution (probability ranging from 0 to 1), logit-link function and binomial error structure (or quasi-binomial error structure, if the residuals of the model were over-dispersed) (Venables and Ripley 1998) were used. Firstly, a GLM was run with seed germination proportion (i.e., number of germinated seeds out of number of viable seeds of each species) as response variable, whereas temperature (T, continuous variable), cold stratification period (CS, continuous variable), PTG (5 level categorical variable) and their interactions were the explanatory variables. Due to the significant PTG groups × T, and PTG groups × CS interaction terms, a second set of GLMs were run, one for each PTG group, in order to assess each PTG germination response to T and CS. GLMs analysis were run with package *lmer* (Bates et al. 2015). Significant differences among category levels were checked with the "Anova" function of package Car (Fox and Weisberg 2019). Ten species were excluded from the statistical analysis: 7 species being characterized by PY dormancy and 3 species that did not germinate at any of the temperature tested.

Finally, the FGPs before and after cold stratification were analyzed by principal component analysis (PCA), using the "Facto-MineR" and "FactoExtra" packages (Lê et al. 2008). Germination data were grouped within the PCA by PTG. Significant differences among PTG were assessed by means of PERMANOVA analysis and Tuckey HSD Post-Hoc test, performed in the PAST environment.

Results

Seed germination response varied from 0 to 100%, across species at the different conditions. Overall, seed germination was promoted by CS, while the effect of T was not significant. The significant negative interaction between T and CS indicate that, overall, after CS the germination increased decreasing the incubation temperature (see Table 1). Germination varied across the five PTG, as confirmed by the ANOVA results reported in Table 2. The significant interaction term between PTG and T indicates that germination response to temperature was significantly different across the five PTGs. No significant interaction between the PTG and CS was found by the ANOVA, indicating that CS had an overall homogeneous and positive effect on seed germination across all the PTGs (Table 1, Table 2, Fig. 4).

Table 1 GLM results of the general model including T, CS, PTG and their interactions. Significant *p*-values are reported in bold.

Variables	Estimate	Std. Error	t-value	*p*-value
Intercept	-0.25136	0.154253	-1.63	n.s.
T	0.000306	0.007608	0.04	n.s.
CS	0.780362	0.17962	4.345	**<0.001**
T × CS	-0.02061	0.007776	-2.651	**<0.001**
PTGs				
DOWN	-1.23231	0.207357	-5.943	**<0.001**
STABLE	-0.9156	0.243069	-3.767	**<0.001**
NEW	-1.53427	0.327232	-4.689	**<0.001**
LOST	-1.71069	0.35031	-4.883	**<0.001**
T × DOWN	0.04318	0.009615	4.491	**<0.001**
T × STABLE	0.03472	0.01131	3.07	**<0.01**
T × NEW	0.031409	0.014788	2.124	**<0.05**

T × LOST	0.043774	0.015729	2.783	**<0.01**
CS × DOWN	0.059482	0.163958	0.363	n.s.
CS × STABLE	0.132579	0.193143	0.686	n.s.
CS × NEW	0.16754	0.253065	0.662	n.s.
CS × LOST	0.012525	0.267225	0.047	n.s.

Table 2 Results of the generalized linear model ANOVA for FGP for different PTG, T, CS and their interactions.

Variables	**Chi-sq.**	**Df**	***p*-value**
PTG	91.241	4	**<0.001**
T × PTG	23.69	4	**<0.001**
CS × PTG	0.755	4	n.s.

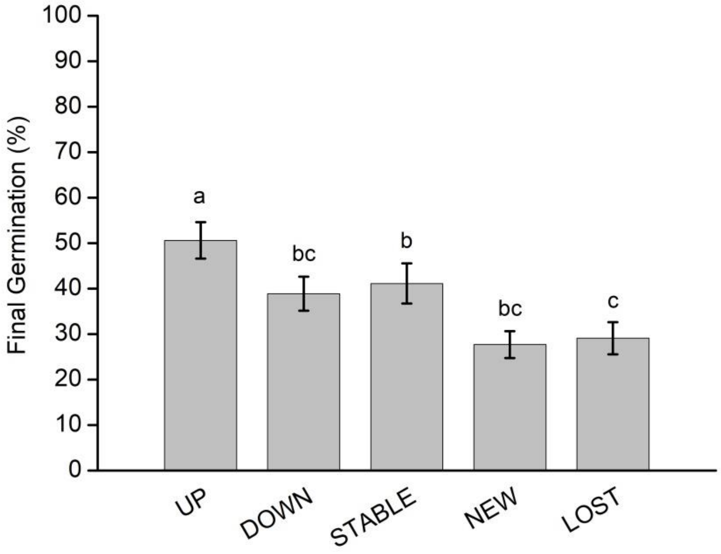
With regard to the single PTG germination traits, PTG UP showed the highest average FGP, statistically different from the other four PTGs (Table 1, Fig. 2). Overall FGP was high for PTG UP, both before (40-50%) and after the CS (40-70%), with no significant differences across species in relation to T (see Table 3). However, after CS the FGP increased decreasing the incubation temperature (significant T × CS reported in Table 3). The species of this group showed the highest FGP at low temperature (5° and 10°C) after CS among the 5 PTGs. Moreover, 5 species of this PTG were found able to germinate also during stratifiacation (with FGP > 50% for 3 species and > 20% for other 2). This trait was shared also by other two species on PTG NEW (with FGP >40%) (see Table S2 for CS germination data).

For all the other PTG groups, T had a slightly positive, but significant effect on FGP in PTG DOWN, STABLE, and LOST, both before and after CS (no significant interaction term between the two variables, see Table 3). In PTG DOWN, STABLE, NEW and LOST, the highest average FGP were found at 20°C and 25°C (Fig. 2), while the lowest at 5° and 10°C. FGP of the PTG DOWN and STABLE, was half way between that showed by PTG UP and the other groups, being between 20% and 60%, depending on the test considered (Fig. 2b and c). NEW and LOST PTGs were characterized by the lowest FGP among the PTGs, being between 10% and 45%, depending on the test considered (Fig. 2d and e).

Table 3 GLM results of each PTGs germination data analyzed singularly. Significant *p*-values are reported in bold.

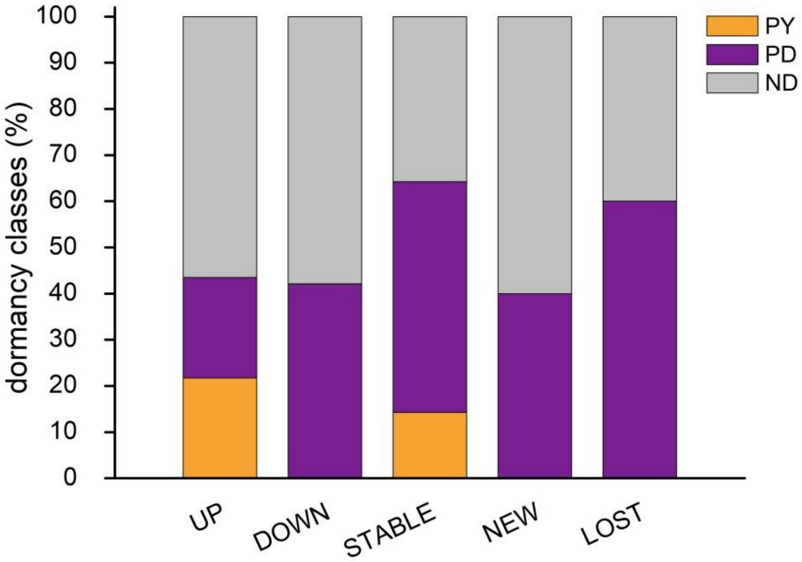
	Variables	Estimate	Std. Error	t-value	*p*-value
UP	T	0.003642	0.009393	0.388	n.s.
	CS	0.914347	0.277559	3.294	**<0.01**
	T × CS	-0.02826	0.014234	-1.985	**<0.05**
DOWN	T	0.041385	0.009612	4.306	**<0.001**
	CS	0.766653	0.266951	2.872	**<0.01**
	T × CS	-0.01658	0.013332	-1.244	n.s.
STABLE	T	0.04272	0.01219	3.505	**<0.001**
	CS	1.18285	0.33766	3.503	**<0.001**
	T × CS	-0.03569	0.01701	-2.098	n.s.
NEW	T	0.01238	0.02089	0.593	n.s.
	CS	0.29201	0.57357	0.509	n.s.
	T × CS	0.0155	0.02872	0.54	n.s.
LOST	T	0.03909	0.01927	2.028	**<0.05**
	CS	0.61078	0.55199	1.107	n.s.
	T × CS	-0.01079	0.02711	-0.398	n.s.

Fig. 2 Mean FGP and standard error values of all the laboratory test for the 5 PTGs. Significant differences among PTGs germination is shown by different letters.



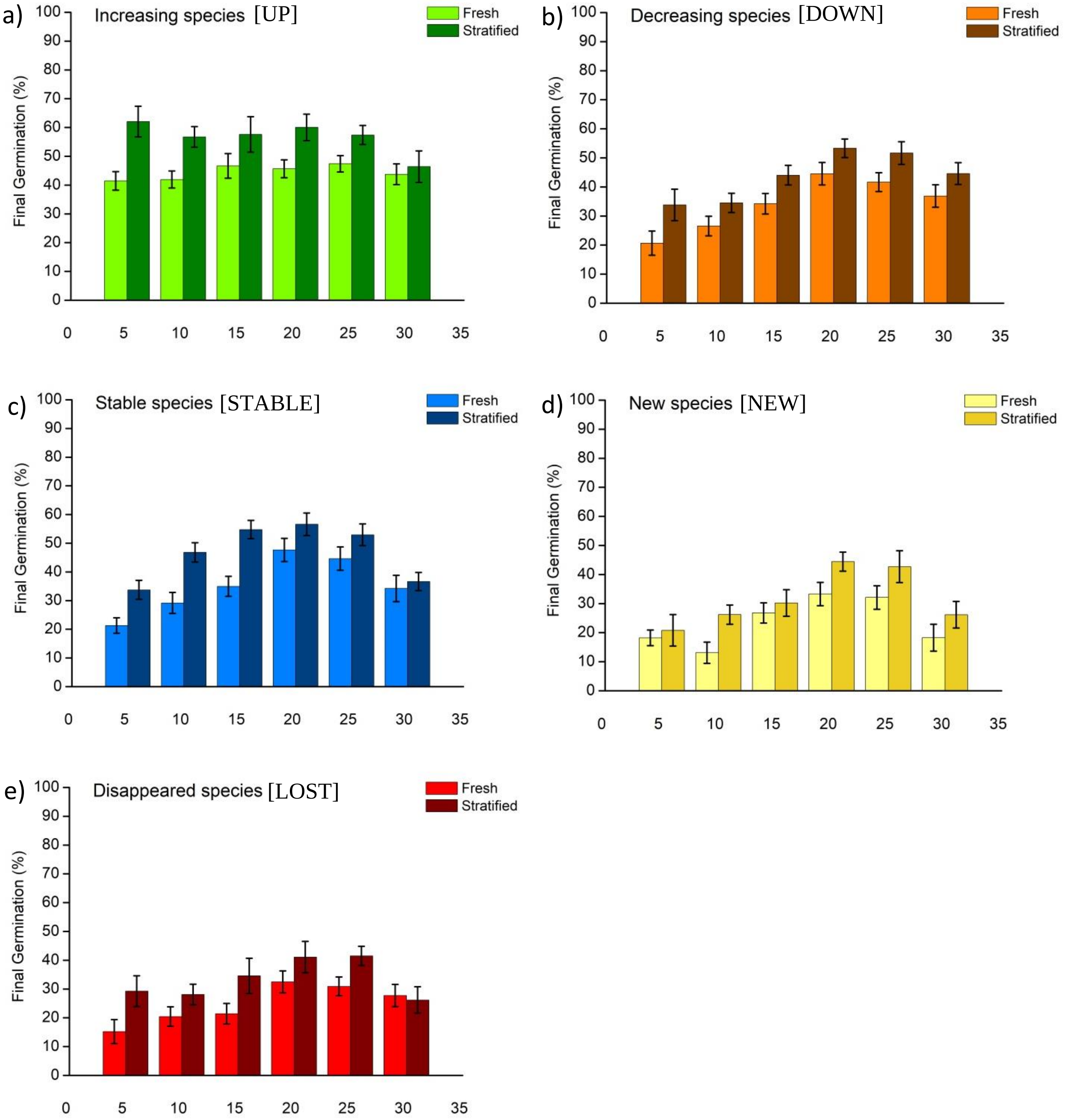
With regard to the dormancy classes, 36 species were non dormant (ND), 23 were physiologically dormant (PD, with different degrees: 11 non-deep, 5 intermediate and 7 deep), 7 species were characterized by physical dormancy (PY) and for 10 species it was not possible to define a clear dormancy pattern (X), likely because the germination requirements were not met (see Table S1 for detailes). Percentage of dormancy classes for every PTG are reported in Fig. 3 (species with undefined dormancy were not included in the graph). Both ND and PD species were found across all the five PTG. The percentage of PD and PY varied across the PTGs. PTG UP resulted carachterized by the lowest percentage of PD species (21%) among the 5 PTG, while PTG LOST had the highest PD species (60%). PY were found only in PTG UP (21%) and PTG STABLE (14%).

Fig. 3 Proportion of dormancy classes for each PTG. PY = Physical dormancy, PD = Physiological dormancy (including n.d. PD, i. PD and d. PD), ND = non dormant.



Finally, the multivariate analysis failed to highlight significant difference of seed germination traits among the five PTGs. No differences among groups were detected by the PERMANOVA analysis and the associated post-hoc tests. In the resulting PCA (not shown), the two main axis explained the 72% of the variability of the data included, but it was also not possible to see a clear pattern of PTG species disposition in the Cartesian space (the PTGs 95% CI ellipses overlapped heavily).

Fig. 4. Average FGP and standard error values of the 5 PTGs, at each temperature tested. Fresh = FGP of Fresh Seeds, Stratified = FGP of seeds after 130 days of cold stratification treatment.



Discussion

Seed germination responses was significantly different across PTGs, with PTG UP consistently showing higher germination compared to that of the other groups and PTG NEW and LOST showing the lowest germination, on average. PTGs differences resulted influenced primary by the germination response to T and its interaction with CS, while CS by its own had a rather homogeneous effect among the groups. Indeed, PTGs responded similarly to CS, which promoted FGP in most of the species, thereby confirming the tendency of alpine plants to germinate better after the winter season (Densmore 1997; Shimono and Kudo 2005; Schwienbacher et al. 2011; Walck et al. 2011; Baskin and Baskin 2014). Despite this, PTG UP included the lowest number of PD species, with no case of deep dormancy, indicating that PD could be a disadvantage in a warmer climate. Following this view, it has been suggested that with shortening of winter (Dong et al. 2010) some seeds may not receive sufficient chilling to satisfy pre-germination vernalization requirements (Fenner et al. 2005), remaining partially dormant (Walck et al. 1997). Consequently, our results highlight that the requirement of cold stratification for seed germination (per se) does not explain plant population dynamics in nature, although some trend may exists. Furthermore, PTG UP also included the highest proportion of PY species (i.e. 5 out of 7 species), suggesting that recruitment from PY seeds might be favoured in a warmer climate. Accordingly, seed germination of physically dormant species was found promoted by both climate warming and heat waves (see Ooi et al. 2009, Ooi et al. 2012). As physical dormancy can be broken by heat (Baskin et al. 2000), warmer soil conditions can alleviate dormancy in physically dormant seeds (Martin et al. 1975; Auld and O'Connell 1991), as well as extreme hot episodes, which are forecasted to become more frequent in the future climate change scenario (Gobiet et al. 2014).

Unlike the response to CS, the five PTGs showed significant different FGP depending on the incubation temperature (T). In particular, as mentioned before, species from PTG UP showed the highest average FGP at all the germination tests, irrespective of the temperature tested. This result, along with the fact that many PTG UP species are ND or n.d. PD, confirm our initial hypothesis that species increasing in nature under a climate change are characterized by high germination at a broad spectrum of temperatures and are less constrained by dormancy patterns (at least PD). Indeed, several species of PTG UP showed high FGP (>85%) irrespective of the temperature tested (*Festuca riccerii*, *Festuca clementei*, *Festuca indigesta*, *Brachipodium genuense*, *Plantago radicata*, etc.). Interestingly, many of those species belongs to the family Poacea. Poaceae, and Graminoids in general have already been reported as one group of plant more favoured by climate change in alpine environments (Venn et al. 2014, Alexander et al. 2016; Porro et al. 2019).

After CS the species of PTG UP were characterized by higher FGP at cold T (5° and 10°C), compared to those of the others groups. Furthermore, 5 species of this group were characterized by the ability to germinate also at 0° in dark condition (i.e. during CS), highlighting an unusual germination strategy that promotes seedling emergence during winter, likely when seeds are still under the snow cover in nature. On one hand, early spring germination may expose seedlings to higher risk of frost (Mercante et al. 2012), but on the other it may be advantageous for alpine species that rely on a short growing season for seedling establishment, i.e. germinating soon after snowmelt, when there is a peak of soil moisture in mountain regions (Molotch et al. 2009; Williams et al. 2009; Bales et al. 2011; Harpold et al. 2015). Seed germination at low temperature early in spring may become more advantageous for seedling survival in a future warmer climate, considering that the timing of snowmelt has already advanced in the past 30 years (Chapin et al. 2005; AMAP 2009). Again, an earlier seedling establishment may also result in a better development of the root system before summer, enhancing the likelihood of survival the hazards of the hot season (Peñaloza 1996, Cavieres and Arroyo 2000).

In the other PTGs seed germination increased, increasing the incubation T, though to a different extent across them. On average, FGP of PTGs NEW and LOST were the lowest at the majority of the temperature tested. Nevertheless, within the two groups are present species with a FGP >75% in at least one of the temperature tested, either before or after CS, depending on the species. Furthermore, there isn't a clear germination response to temperature in both groups, e.g. they include species that germinate either at low or high T, before and after CS. However, in PTG NEW (similarly to PTG UP) are present 2 species (*Rhinantus angustifolius* and *Seseli libanotis*) that were able to germinate during CS (FGP > 45% at 0°C). This trait strengthens the hypothesis that this germination strategy could be advantageous in the context of climate change, as it was mainly observed for increasing and incoming plant species within the GLORIA study sites (i.e. PTG UP and NEW). Unlike PTG NEW and UP, there was only one species able to germinate during CS in PTG LOST (*Rinanthus glacialis*, with a FGP < 25%).

With regard to the other PTGs, both PTG STABLE and DOWN were characterized by similar FGP, with an average seed germination optimum at hot T and a positive effect of CS, though none of these two PTGs included species able to germinate during CS. This germination behaviour seems to reflect the strategy observed in many alpine species (e.g. Kibe and Mazusawa 1994; Cavieres and Arroyo 2000; Shimono and Kudo 2005; Baskin and Baskin 2014), which was considered an adaptation to prevent early spring or autumn germination when a high likelihood of frost may result in a low probability of seedling establishment (Cavieres and Arroyo 2000). However, according to our results a delayed germination, synchronized with higher temperature may not be advantageous with warming, as it elicit seedling emergence in periods of the year becoming less suitable. Indeed, climatic models discussed in Calanca (2007) indicate that a decrement in the frequency of wet days of about 20% has to be expected in Alpine environments, as well as an increment of droughts frequency from about 15% to more than 50%. Additionally, there are also expected a substantial increase in the likelihood of heat waves (Beniston and Stephenson 2004, Beniston and Diaz 2004, Schär et al. 2004; Meehl and Tebaldi 2004). Both drought and heat waves, besides the gradually increasing temperature, may heavily affect plant recruitment and growth (Thuiller et al. 2005; Engler et al. 2011, Orsenigo et al. 2014; Orsenigo et al. 2015). Moreover, these two PTG include a higher proportion of PD species than PTG UP (42% and 50% respectively), indicating that with shortening of winter season (Dong et al. 2010), these species may not overcome seed dormancy.

Concluding remarks

The results presented here provide first insights for interpreting alpine plant population dynamics in the context of climate change, focussing on regenerative traits. Overall, the alpine species included in this study germinated better at warm conditions and after the cold stratification treatment. However, species that are increasing in nature (PTG UP) could germinate at a broader spectrum of environmental conditions than those of the other groups, i.e. under snow cover and with high FGP at low temperature, especially after CS. These traits suggest that germination during early spring, right after snowmelt, may be an advantage in a warmer climate, for example, promoting seedling emergence when water is more available and drought/heat hazards are low. On the other hand, seed germination programmed to occur later in spring, when temperature increse (typical of PTG STABLE and DOWN), may expose the young seedlings to higher risks of drought and/or heat stress in a warmer climate, affecting recruitment success.

Aknowledgements

I greatfully acknowledge the researchers of the University of Innsbruck (AU), the University of L’Aquila and the University of Granada, along with the members of the “Alpine seed conservation and research network” (http://www.alpineseedconservation.eu/), that were involved in the seed collection campaign, and that make this project possible.

References

- Alexander, J. M., Lembrechts, J. J., Cavieres, L. A., Daehler, C., Haider, S., Kueffer, C., ... and Rew, L. J. (2016). Plant invasions into mountains and alpine ecosystems: current status and future challenges. Alpine Botany, 126(2), 89-103.
- AMAP. (2009). AMAP 2009 Update on Selected Climate Issues of Concern.
- Auld, T. D., and O'Connell, M. A. (1991). Predicting patterns of post-fire germination in 35 eastern Australian Fabaceae. Australian Journal of Ecology, 16(1), 53-70.
- Bales, R. C., Hopmans, J. W., O'Geen, A. T., Meadows, M., Hartsough, P. C., Kirchner, P., ... and Beaudette, D. (2011). Soil moisture response to snowmelt and rainfall in a Sierra Nevada mixed-conifer forest. Vadose Zone Journal, 10(3), 786-799.
- Baskin, J. M., and Baskin, C. C. (2000). Evolutionary considerations of claims for physical dormancy-break by microbial action and abrasion by soil particles. Seed Science Research, 10(4), 409-413.
- Baskin, C.C. and Baskin, J.M. (2014). Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, New York, NY, US.
- Beniston, M., and Stephenson, D. B. (2004). Extreme climatic events and their evolution under changing climatic conditions. Global and planetary change, 44(1-4), 1-9.
- Beniston, M., and Diaz, H. F. (2004). The 2003 heat wave as an example of summers in a greenhouse climate? Observations and climate model simulations for Basel, Switzerland. Global and planetary change, 44(1-4), 73-81.
- Bernareggi, G., Carbognani, M., Petraglia, A., and Mondoni, A. (2015). Climate warming could increase seed longevity of alpine snowbed plants. Alpine Botany, 125(2), 69-78.
- Bernareggi, G., Carbognani, M., Mondoni, A., and Petraglia, A. (2016). Seed dormancy and germination changes of snowbed species under climate warming: the role of pre-and post-dispersal temperatures. Annals of botany, 118(3), 529-539.
- Billings, W. D., and Mooney, H. A. (1968). The ecology of arctic and alpine plants. Biological reviews, 43(4), 481-529.
- Briceño, V. F., Hoyle, G. L., and Nicotra, A. B. (2015). Seeds at risk: how will a changing alpine climate affect regeneration from seeds in alpine areas?. Alpine Botany, 125(2), 59-68.
- Calanca, P. (2007). Climate change and drought occurrence in the Alpine region: how severe are becoming the extremes?. Global and Planetary Change, 57(1-2), 151-160.

- Cavieres, L. A., and Arroyo, M. T. (2000). Seed germination response to cold stratification period and thermal regime in Phacelia secunda (Hydrophyllaceae)–altitudinal variation in the Mediterranean Andes of central Chile. Plant Ecology, 149(1), 1-8.
- Chapin, F. S., Sturm, M., Serreze, M. C., McFadden, J. P., Key, J. R., Lloyd, A. H., ... and Beringer, J. (2005). Role of land-surface changes in Arctic summer warming. science, 310(5748), 657-660.
- Dalgleish, H. J., Koons, D. N., and Adler, P. B. (2010). Can life-history traits predict the response of forb populations to changes in climate variability?. Journal of Ecology, 98(1), 209-217.
- Densmore, R. V. (1997). Effect of day length on germination of seeds collected in Alaska. American Journal of Botany, 84(2), 274-278.
- Díaz, S., Kattge, J., Cornelissen, J. H., Wright, I. J., Lavorel, S., Dray, S., ... and Garnier, E. (2016). The global spectrum of plant form and function. Nature, 529(7585), 167.
- Diaz, H. F., Bradley, R. S., and Ning, L. (2014). Climatic changes in mountain regions of the American Cordillera and the tropics: historical changes and future outlook. Arctic, Antarctic, and Alpine Research, 46(4), 735-743.
- Dong, W., Jiang, Y., Yang, S. (2010). Response of the starting dates and the lengths of seasons in mainland China to global warming. Climatic Change 99: 81–91.
- Donohue, K., Rubio de Casas, R., Burghardt, L., Kovach, K., and Willis, C. G. (2010). Germination, postgermination adaptation, and species ecological ranges. Annual review of ecology, evolution, and systematics, 41, 293-319.
- Dürr, C., Dickie, J. B., Yang, X. Y., and Pritchard, H. W. (2015). Ranges of critical temperature and water potential values for the germination of species worldwide: contribution to a seed trait database. Agricultural and forest meteorology, 200, 222-232.
- Engler, R., Randin, C. F., Thuiller, W., Dullinger, S., Zimmermann, N. E., Araújo, M. B., ... and Choler, P. (2011). 21st century climate change threatens mountain flora unequally across Europe. Global Change Biology, 17(7), 2330-2341.
- Fenner, M. K., Fenner, M., and Thompson, K. (2005). The ecology of seeds. Cambridge University Press.
- Fernández-Pascual, E., Jiménez-Alfaro, B., Hájek, M., Díaz, T. E., and Pritchard, H. W. (2015). Soil thermal buffer and regeneration niche may favour calcareous fen resilience to climate change. Folia Geobotanica, 50(4), 293-301.

- Funk, J. L., Standish, R. J., Stock, W. D., and Valladares, F. (2016). Plant functional traits of dominant native and invasive species in mediterranean-climate ecosystems. Ecology, 97(1), 75-83.
- Gobiet, A., Kotlarski, S., Beniston, M., Heinrich, G., Rajczak, J., and Stoffel, M. (2014). 21st century climate change in the European Alps—a review. Science of the Total Environment, 493, 1138-1151.
- Gottfried, M., Pauli, H., Futschik, A., Akhalkatsi, M., Barančok, P., Alonso, J. L. B., ... and Grabherr, G. (2012). Continent-wide response of mountain vegetation to climate change. Nature Climate Change, 2(2), 111-115.
- Grabherr, G., Gottfried, M., Gruber, A., and Pauli, H. (1995). Patterns and current changes in alpine plant diversity. In Arctic and alpine biodiversity: patterns, causes and ecosystem consequences (pp. 167-181). Springer Berlin Heidelberg.
- Grubb, P. J. (1977). The maintenance of species-richness in plant communities: the importance of the regeneration niche. Biological reviews, 52(1), 107-145.
- Harpold, A. A., Molotch, N. P., Musselman, K. N., Bales, R. C., Kirchner, P. B., Litvak, M., and Brooks, P. D. (2015). Soil moisture response to snowmelt timing in mixed-conifer subalpine forests. Hydrological Processes, 29(12), 2782-2798.
- Hoyle, G. L., Venn, S. E., Steadman, K. J., Good, R. B., McAuliffe, E. J., Williams, E. R., and Nicotra, A. B. (2013). Soil warming increases plant species richness but decreases germination from the alpine soil seed bank. Global Change Biology, 19(5), 1549-1561.
- IPCC (2014). Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. IPCC, Geneva, Switzerland.
- Jiménez-Alfaro, B., Silveira, F. A., Fidelis, A., Poschlod, P., and Commander, L. E. (2016). Seed germination traits can contribute better to plant community ecology. Journal of Vegetation Science, 27(3), 637-645.
- Kibe, T., and Masuzawa, T. (1994). Seed germination and seedling growth of Carex doenitzii growing on alpine zone of Mt. Fuji. Journal of Plant Research, 107(1), 23-27.
- Keddy, P. A. (1992). Assembly and response rules: two goals for predictive community ecology. Journal of Vegetation Science, 3(2), 157-164.
- Klanderud, K., and Birks, H. J. B. (2003). Recent increases in species richness and shifts in altitudinal distributions of Norwegian mountain plants. The Holocene, 13(1),
- Larson, J. E., and Funk, J. L. (2016). Regeneration: an overlooked aspect of trait-based plant community assembly models. Journal of Ecology, 104(5), 1284-1298.

- Lavorel, S., and Garnier, É. (2002). Predicting changes in community composition and ecosystem functioning from plant traits: revisiting the Holy Grail. Functional ecology, 16(5), 545-556.
- Lenoir J, Gégout JC, Marquet PA, De Ruffray P, Brisse H (2008) Significant upward shift in plant species optimum elevation during the 20th century. Science 320:1768-1771
- Lloret, F., Penuelas, J., and Estiarte, M. (2004). Experimental evidence of reduced diversity of seedlings due to climate modification in a Mediterranean-type community. Global Change Biology, 10(2), 248-258.
- Marcante, S., Erschbamer, B., Buchner, O., and Neuner, G. (2014). Heat tolerance of early developmental stages of glacier foreland species in the growth chamber and in the field. Plant ecology, 215(7), 747-758.
- Martin, A. C. (1946). The comparative internal morphology of seeds. The American Midland Naturalist, 36(3), 513-660.
- Martin, R. E., Miller, R. L., and Cushwa, C. T. (1975). Germination response of legume seeds subjected to moist and dry heat. Ecology, 56(6), 1441-1445.
- McGill, B. J., Enquist, B. J., Weiher, E., and Westoby, M. (2006). Rebuilding community ecology from functional traits. Trends in ecology and evolution, 21(4), 178-185.
- Meehl, G. A., and Tebaldi, C. (2004). More intense, more frequent, and longer lasting heat waves in the 21st century. Science, 305(5686), 994-997
- Milbau, A., Graae, B. J., Shevtsova, A., and Nijs, I. (2009). Effects of a warmer climate on seed germination in the subarctic. Annals of Botany, 104(2), 287-296.
- Molotch, N. P., Brooks, P. D., Burns, S. P., Litvak, M., Monson, R. K., McConnell, J. R., and Musselman, K. (2009). Ecohydrological controls on snowmelt partitioning in mixed-conifer sub-alpine forests. Ecohydrology: Ecosystems, Land and Water Process Interactions, Ecohydrogeomorphology, 2(2), 129-142.
- Mondoni, A., Rossi, G., Orsenigo, S., and Probert, R. J. (2012). Climate warming could shift the timing of seed germination in alpine plants. Annals of Botany, 110(1), 155-164.
- Mondoni, A., Pedrini, S., Bernareggi, G., Rossi, G., Abeli, T., Probert, R. J., ... and Orsenigo, S. (2015). Climate warming could increase recruitment success in glacier foreland plants. Annals of Botany, 116(6), 907-916.
- Neilson, R. P., Pitelka, L. F., Solomon, A. M., Nathan, R. A. N., Midgley, G. F., Fragoso, J. M., ... and Thompson, K. E. N. (2005). Forecasting regional to global plant migration in response to climate change. Bioscience, 55(9), 749-759.

- Nogués-Bravo, D., Araújo, M. B., Errea, M. P., and Martinez-Rica, J. P. (2007). Exposure of global mountain systems to climate warming during the 21st Century. Global Environmental Change, 17(3), 420-428.
- Ooi, M. K., Auld, T. D., and Denham, A. J. (2009). Climate change and bet-hedging: interactions between increased soil temperatures and seed bank persistence. Global Change Biology, 15(10), 2375-2386.
- Ooi, M. K. (2012). Seed bank persistence and climate change. Seed Science Research, 22(S1), S53-S60.
- Ordoñez-Salanueva, C. A., Seal, C. E., Pritchard, H. W., Orozco-Segovia, A., Canales-Martínez, M., and Flores-Ortiz, C. M. (2015). Cardinal temperatures and thermal time in Polaskia Backeb (Cactaceae) species: effect of projected soil temperature increase and nurse interaction on germination timing. Journal of Arid Environments, 115, 73-80.
- Orru, M., Mattana, E., Pritchard, H. W., and Bacchetta, G. (2012). Thermal thresholds as predictors of seed dormancy release and germination timing: altitude-related risks from climate warming for the wild grapevine Vitis vinifera subsp. sylvestris. Annals of Botany, 110(8), 1651-1660.
- Orsenigo, S., Mondoni, A., Rossi, G., Abeli, T. (2014) Some like it hot and some like it cold, but not too much: plant responses to climate extremes. Plant Ecology 215:677-688
- Orsenigo, S., Abeli, T., Rossi, G. et al (2015) Effects of autumn and spring heat waves on seed germination of high mountain plants. PLOS One 10(7): e0133626 doi:10.1371/journal.pone.0133626
- Parolo, G., and Rossi, G. (2008). Upward migration of vascular plants following a climate warming trend in the Alps. Basic and Applied Ecology, 9(2), 100-107.
- Pauli, H., Gottfried, M., Dullinger, S., Abdaladze, O., Akhalkatsi, M., Alonso, J. L. B., ... and Ghosn, D. (2012). Recent plant diversity changes on Europe’s mountain summits. Science, 336(6079), 353-355.
- Pauli, H., Gottfried, M., Lamprecht, A., Niessner, S., Rumpf, S., Winkler, M., ... and Grabherr, G. (2015). The GLORIA field manual—standard Multi-Summit approach, supplementary methods and extra approaches. Vienna: GLORIA-Coordination, Austrian Academy of Sciences and University of Natural Resources and Life Sciences.
- Pearson, R. G. (2006). Climate change and the migration capacity of species. Trends in ecology and evolution, 21(3), 111-113.

- Peñaloza, A. 1996. Consideraciones microclimáticas en la sobrevivencia de semillas y plántulas de Kageneckia angustifolia Don una especie del bosque montano de Chile central. Tesis de Magister, Facultad de Ciencias, Universidad de Chile.
- Poschlod, P., Abedi, M., Bartelheimer, M., Drobnik, J., Rosbakh, S., and Saatkamp, A. (2013). Seed ecology and assembly rules in plant communities. Vegetation ecology, 164-202.
- Porro, F., Tomaselli, M., Abeli, T., Gandini, M., Gualmini, M., Orsenigo, S., ... and Carbognani, M. (2019). Could plant diversity metrics explain climate-driven vegetation changes on mountain summits of the GLORIA network?. Biodiversity and Conservation, 1-22.
- R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
- Read, Q. D., Moorhead, L. C., Swenson, N. G., Bailey, J. K., and Sanders, N. J. (2014). Convergent effects of elevation on functional leaf traits within and among species. Functional ecology, 28(1), 37-45.
- Saatkamp, A., Cochrane, A., Commander, L., Guja, L. K., Jimenez-Alfaro, B., Larson, J., ... and Dalziell, E. L. (2019). A research agenda for seed-trait functional ecology. New Phytologist, 221(4), 1764-1775.
- Seal, C. E., Daws, M. I., Flores, J., Ortega-Baes, P., Galíndez, G., León-Lobos, P., ... and Ordoñez-Salanueva, C. A. (2017). Thermal buffering capacity of the germination phenotype across the environmental envelope of the Cactaceae. Global change biology, 23(12), 5309-5317.
- Schär, C., Vidale, P. L., Lüthi, D., Frei, C., Häberli, C., Liniger, M. A., and Appenzeller, C. (2004). The role of increasing temperature variability in European summer heatwaves. Nature, 427(6972), 332.
- Shimono, Y., and Kudo, G. (2005). Comparisons of germination traits of alpine plants between fellfield and snowbed habitats. Ecological Research, 20(2), 189-197.
- Schwienbacher, E., Marcante, S., and Erschbamer, B. (2010). Alpine species seed longevity in the soil in relation to seed size and shape–A 5-year burial experiment in the Central Alps. Flora-Morphology, Distribution, Functional Ecology of Plants, 205(1), 19-25.
- Schwienbacher, E., Navarro-Cano, J. A., Neuner, G., and Erschbamer, B. (2011). Seed dormancy in alpine species. Flora-Morphology, Distribution, Functional Ecology of Plants, 206(10), 845-856.

- Shevtsova, A., Graae, B. J., Jochum, T., Milbau, A. N. N., Kockelbergh, F., Beyens, L., and Nijs, I. (2009). Critical periods for impact of climate warming on early seedling establishment in subarctic tundra. Global Change Biology, 15(11), 2662-2680.
- Silveira, F. A. O. (2013). Sowing seeds for the future: the need for establishing protocols for the study of seed dormancy. Acta Botanica Brasilica, 27(2), 264-269.
- Silvertown, J., and Charlesworth, D. (2001). Introduction to plant population biology, 4th edn Oxford. UK: Wiley-Blackwell.
- Thuiller, W., Lavorel, S., Araujo, M.B., Sykes, M.T., Colin Prentice. (2005). Climate change threats to plant diversity in Europe. PNAS, 102 (23), 8245-8250.
- Tudela-Isanta, M., Fernández-Pascual, E., Wijayasinghe, M., Orsenigo, S., Rossi, G., Pritchard, H. W., and Mondoni, A. (2018a). Habitat-related seed germination traits in alpine habitats. Ecology and evolution, 8(1), 150-161.
- Tudela-Isanta, M., Ladouceur, E., Wijayasinghe, M., Pritchard, H. W., and Mondoni, A. (2018b). The seed germination niche limits the distribution of some plant species in calcareous or siliceous alpine bedrocks. Alpine Botany, 128(1), 83-95.
- Valladares, F., Matesanz, S., Guilhaumon, F., Araújo, M. B., Balaguer, L., Benito-Garzón, M., ... and Nicotra, A. B. (2014). The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecology letters, 17(11), 1351-1364.
- Venables, W. N., and Ripley, B. D. (1998). Modern Applied Statistics with S-Plus,(3rd corrected printing).
- Venn, S., Pickering, C., and Green, K. (2014). Spatial and temporal functional changes in alpine summit vegetation are driven by increases in shrubs and graminoids. AoB Plants, 6.
- Way, M. J. (2003). Collecting seed from non-domesticated plants for long-term conservation. Seed conservation: turning science into practice, 165-201.
- Walck, J. L., Baskin, J. M., and Baskin, C. C. (1997). A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of Solidago (Asteraceae). 1. Germination phenology and effect of cold stratification on germination. Seed Science Research, 7(1), 47-58.
- Walck, J. L., Hidayati, S. N., Dixon, K. W., Thompson, K. E. N., and Poschlod, P. (2011). Climate change and plant regeneration from seed. Global Change Biology, 17(6), 2145-2161.
- Walther GR, Beißner S, Burga CA (2005) Trends in the upward shift of alpine plants. Journal of Vegetation Science 16:541-548

- Weiher, E., Van Der Werf, A., Thompson, K., Roderick, M., Garnier, E., and Eriksson, O. (1999). Challenging Theophrastus: a common core list of plant traits for functional ecology. Journal of vegetation science, 10(5), 609-620.
- Williams, C. J., McNamara, J. P., and Chandler, D. G. (2009). Controls on the temporal and spatial variability of soil moisture in a mountainous landscape: the signature of snow and complex terrain. Hydrology and Earth System Sciences, 13(7), 1325-133

Appendix

Table S1 Target species list. Dormancy codes: ND = non-dormant; PY = physical dormancy; n.d. PD = non-deep physiological dormancy; i. PD = intermediate physiological dormancy; d. Deep physiological dormancy.

ID code	Species	TR	PTG	Dormancy
Ali_spin	*Alyssum spinosum* L.	Es-Sne	UP	ND
Hol_cae	*Holcus caespitosus* Boiss.	Es-Sne	UP	ND
Fes_cle	*Festuca clementei* Boiss.	Es-Sne	UP	ND
Fes_ind	*Festuca indigesta* Boiss. subsp. indigesta	Es-Sne	UP	ND
Pla_rad	*Plantago radicata* Hoffmanns. and Link	Es-Sne	UP	ND
Alc_alp	*Alchemilla alpina* L.	It-Nap	UP	n.d. PD
Fes_ric	*Festuca riccerii* Foggi and Gr. Rossi	It-Nap	UP	ND
Jun_tri	*Juncus trifidus* L.	It-Nap	UP	ND
Pol_bys	*Polygonum bistorta* L.	It-Nap	UP	n.d. PD
Bra_gen	*Brachypodium genuense* (DC.) Roem. and Schult.	It-Nap	UP	ND
Hel_oel	*Helianthemum oelandicum* (L.) DC.	It-Maj	UP	X
Ach_bar	*Achillea barrelieri* Ten.	It-Maj	UP	ND
Gen_ani	*Gentianella anisodonta* (Borbás) Á.Löve and D.Löve	It-Ado	UP	i. PD
Ach_cla	*Achillea clavennae* L.	It-Ado	UP	ND
Min_ver	*Minuartia verna* (L.) Hiern subsp. *verna*	It-Ado	UP	i. PD
Luz_mul	*Luzula multiflora* (Ehrh.) Lej.	It-Tex	UP	ND
Ver_bel	*Veronica bellidioides* L.	It-Tex	UP	ND
Phy_bet	*Phyteuma betonicifolium* Vill.	It-Tex	UP	ND
Phy_em2	*Phyteuma hemisphaericum* L.	It-Tex	UP	n.d. PD
Gen_tin	*Genista tinctoria* L.	It-Nap	UP	PY
Ant_vul	*Anthyllis vulneraria* subsp. *pulchella* (Vis.) Bornm	It-Maj	UP	PY
Tri_alp	*Trifolium alpinum* L.	It-Tex	UP	PY
Oxy_jac	*Oxytropis jacquinii* Bunge	It-Ado	UP	PY
Oxy_cam	*Oxytropis campestris* (L.) DC.	It-Maj	UP	PY
Sal_ret	*Salix retusa* L.	It-Ado	UP	X
Sal_ret2	*Salix retusa* L.	It-Nap	UP	X
Tri_gla	*Trisetum glaciale* (Bory) Boiss.	Es-Sne	DOWN	ND
Are_tet	*Arenaria tetraquetra* L. subsp. *amabilis* (Bory) H. Lindb.	Es-Sne	DOWN	ND
Gal_pyr	*Galium pyrenaicum* Gouan	Es-Sne	DOWN	d. PD
Sid_gla	*Sideritis glacialis* Boiss.	Es-Sne	DOWN	ND
Car_car	*Carduus carlinoides* Gouan subsp. hispanicus (Kazmi) Franco	Es-Sne	DOWN	n.d. PD
Phy_hem	*Phyteuma hemisphaericum* L.	It-Nap	DOWN	ND

Leo_pyr	*Leontodon pyrenaicus* Gouan subsp. *helveticus* (Mérat) Finch and P.D. Sell	It-Nap	DOWN	ND
Sil_aca	*Silene acaulis* subsp. *bryoides* (Jord.) Nyman	It-Nap	DOWN	ND
Car_sem	*Carex sempervirens* Vill.	It-Nap	DOWN	X
Luz_lut	*Luzula lutea* (All.) DC.	It-Nap	DOWN	n.d. PD
Hel_num	*Helianthemum nummularium* subsp. *grandiflorum* (Scop.) Schinz and Thell.	It-Maj	DOWN	X
Are_gran	*Arenaria grandiflora* L.	It-Maj	DOWN	i. PD
Sax_opp	*Saxifraga oppositifolia* L.	It-Maj	DOWN	d. PD
Ast_alp	*Aster alpinus* L.	It-Maj	DOWN	ND
Agr_alp	*Agrostis alpina* Scop.	It-Ado	DOWN	n.d. PD
Luz_lu2	*Luzula lutea* (All.) DC.	It-Ado	DOWN	n.d. PD
Leo_hel	*Leontodon pyrenaicus* Gouan subsp. *helveticus* (Mérat) Finch and P.D. Sell	It-Ado	DOWN	ND
Car_atr	*Carex atrata* L. subsp. *atrata*	It-Ado	DOWN	ND
Pri_glu	*Primula glutinosa* Wulfen	It-Tex	DOWN	n.d. PD
Cam_bar	*Campanula barbata* L.	It-Tex	DOWN	ND
Leo_hi2	*Leontodon hispidus* L.	It-Tex	DOWN	ND
Des_fle	*Deschampsia flexuosa* (L.) Trin. subsp. *iberica* Rivas Martínez	Es-Sne	STABLE	d. PD
Cha_gla	*Chaenorhinum glareosum* (Boiss.) Willk.	Es-Sne	STABLE	i. PD
Thy_ser	*Thymus serpylloides* Bory subsp. *serpylloides*	Es-Sne	STABLE	i. PD
Vac_myr	*Vaccinium myrtillus* L.	It-Nap	STABLE	ND
Hyp_ric	*Hypericum richeri* Vill.	It-Nap	STABLE	ND
Pla_ser	*Plantago maritima* subsp. *serpentina* (All.) Arcang.	It-Nap	STABLE	ND
Arm_can	*Armeria canescens* (Host) Boiss.	It-Maj	STABLE	ND
Pol_viv	*Polygonum viviparum* L.	It-Ado	STABLE	ND
Car_rup	*Carex rupestris* All.	It-Ado	STABLE	d. PD
Min_sed	*Minuartia sedoides* (L.) Hiern	It-Tex	STABLE	n.d. PD
Ore_dis	*Oreochloa disticha* (Wulfen) Link.	It-Tex	STABLE	d. PD
Geu_mon	*Geum montanum* L.	It-Tex	STABLE	n.d. PD
Car_cur	*Carex curvula* All.	It-Tex	STABLE	X
Lot_cor	*Lotus corniculatus* L.	It-Nap	STABLE	PY
Ane_bal	*Anemone baldensis* L.	It-Ado	STABLE	X
Gen_rad	*Genista radiata* (L.) Scop.	It-Nap	STABLE	PY
Log_arv	*Logfia arvensis* (L.) Holub	Es-Sne	NEW	ND
Rhi_ang	*Rhinanthus angustifolius* C.C. Gmel.	It-Nap	NEW	ND
Ses_lib	*Seseli libanotis* (L.) W.D.J. Koch	It-Nap	NEW	ND
Eup_Sal	*Euphrasia salisburgensis* Funck ex Hoppe.	It-Maj	NEW	X
Sed_atr	*Sedum atratum* L.	It-Maj	NEW	d. PD

Rho_fer	*Rhododendron ferrugineum* L.	It-Tex	NEW	n.d. PD
Res_com	*Reseda complicata* Bory	Es-Sne	LOST	ND
Arm_mar	*Armeria marginata* (Levier) Bianchini	It-Nap	LOST	ND
Phy_orb	*Phyteuma orbiculare* L.	It-Nap	LOST	d. PD
Aco_lyc	*Aconitum lycoctonum* subsp. *neapolitanum* (Ten.) Nyman	It-Ado	LOST	n.d. PD
Sol_vir	*Solidago virgaurea* L.	It-Ado	LOST	n.d. PD
Rin_gla	*Rhinanthus glacialis* Personnat	It-Tex	LOST	X

Table S2 FGP of all the Target species used for the data analysis in this chapter. Fresh seed = FGP of non stratified seeds. CS = FGP recorded during sratification treatment (0°C, 24h darkness condition). after CS = FGP of stratified seeds.

PTG	**species code**	**fresh seed**						**CS**	**after CS**					
		5°	**10°**	**15°**	**20°**	**25°**	**30°**	**0°**	**5°**	**10°**	**15°**	**20°**	**25°**	**30°**
UP	Ali_spin	67.9	41.3	72.0	29.3	44.0	34.7	27.2	84.8	86.3	79.3	74.3	69.4	86.8
UP	Hol_cae	7.1	22.1	60.0	53.3	52.2	90.2	0.0	4.8	5.6	42.8	92.6	100	77.0
UP	Fes_cle	66.7	98.7	98.7	98.7	100	100	20.5	90.0	100	100	100	100	100
UP	Fes_ind	100	92.1	94.7	100	93.3	82.2	83.6	68.3	73.5	75.8	86.7	65.6	19.0
UP	Pla_rad	86.7	96.0	89.3	96.0	97.3	89.3	19.9	86.5	97.9	96.5	100	86.7	81.3
UP	Alc_alp	1.3	1.3	2.7	8.0	5.3	10.7	0.0	52.5	30.3	38.8	29.6	28.0	26.7
UP	Fes_ric	80.7	88.0	89.3	77.3	93.0	80.0	80.9	38.4	60.6	37.8	50.6	36.7	40.5
UP	Jun_tri	91.2	12.3	2.7	1.3	2.7	1.3	0.4	96.0	93.1	94.7	95.9	91.9	39.3
UP	Pol_bys	9.3	0.0	0.0	0.0	2.7	16.3	54.9	79.3	84.8	63.4	42.6	63.8	59.5
UP	Bra_gen	18.7	31.6	27.3	66.7	82.9	84.0	0.0	79.9	75.7	78.5	79.7	82.3	75.9
UP	Hel_oel	17.3	18.9	25.3	16.0	17.3	13.3	5.5	4.5	11.0	23.1	24.7	29.4	31.5
UP	Ach_bar	18.7	80.0	74.3	98.7	97.3	98.7	0.0	98.7	100	93.2	100	100	98.7
UP	Gen_ani	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.8	8.1	0.0	1.3
UP	Ach_cla	6.4	24.9	42.6	50.7	70.3	70.7	0.8	79.5	81.7	79.3	89.6	100	89.6
UP	Min_ver	0.0	5.3	8.0	6.7	6.6	2.7	3.2	1.4	0.0	0.0	5.8	7.7	4.1
UP	Luz_mul	34.7	89.3	90.7	97.3	98.7	52.0	0.0	41.3	86.7	96.0	97.3	91.8	21.3
UP	Ver_bel	89.3	14.7	9.3	0.0	6.7	0.0	5.1	100	1.3	0.0	1.4	1.3	0.0
UP	Phy_bet	90.7	67.3	81.9	61.3	24.0	1.3	0.7	83.4	56.3	35.8	19.1	25.5	20.2
UP	Phy_e2	1.3	13.3	18.7	6.7	6.7	4.0	3.5	90.3	74.0	57.0	43.1	10.6	9.7
DOWN	Tri_gla	5.6	38.9	12.5	8.2	11.3	15.3	0.0	24.8	20.6	0.0	0.0	18.9	11.1
DOWN	Are_tet	71.2	98.4	100	98.3	100	98.3	6.2	28.8	50.7	98.7	95.6	95.8	65.7
DOWN	Gal_pyr	0.0	1.3	0.0	0.0	4.0	2.7	2.7	1.9	3.1	3.2	0.0	7.4	1.7
DOWN	Sid_gla	6.9	10.7	74.7	87.7	64.7	77.5	0.0	12.5	10.9	74.9	79.5	74.9	52.0
DOWN	Car_car	28.9	26.9	26.7	51.0	48.2	51.1	6.7	9.8	7.8	24.5	62.3	61.4	64.7
DOWN	Phy_he	44.0	57.3	27.8	9.3	5.4	1.3	10.5	60.8	58.8	50.6	43.9	55.0	61.5

DOWN	Leo_pyr	38.1	69.4	86.9	95.2	98.3	69.3	2.6	100	92.4	92.9	95.2	100	94.0
DOWN	Sil_aca	1.4	8.0	35.5	85.2	93.2	94.6	0.4	22.6	73.0	97.4	97.3	94.7	93.7
DOWN	Car_sem	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2	0.0	0.0
DOWN	Luz_lut	58.7	4.0	0.0	27.2	0.0	12.0	3.7	69.0	37.2	33.0	37.6	22.6	44.4
DOWN	Hel_num	12.0	8.0	14.7	20.0	10.7	8.0	1.8	9.6	6.9	10.8	17.9	8.0	9.2
DOWN	Are_gra	1.3	1.3	6.6	6.4	5.3	1.3	0.7	4.0	2.7	2.7	2.7	0.0	0.0
DOWN	Sax_opp	13.1	4.0	4.0	1.3	2.6	0.0	0.9	22.7	8.7	5.4	4.0	1.3	1.3
DOWN	Ast_alp	20.0	62.2	77.8	68.9	77.8	48.9	3.0	55.6	64.3	57.2	77.8	68.8	91.7
DOWN	Agr_alp	10.8	13.2	19.6	17.3	27.6	28.4	0.9	61.8	63.6	59.0	59.9	72.0	48.7
DOWN	Luz_lu2	24.0	4.0	1.3	54.7	5.3	2.7	0.0	64.2	5.6	7.6	72.3	16.3	2.7
DOWN	Leo_hel	80.9	53.9	91.9	84.0	93.2	78.7	6.9	77.7	76.8	92.9	97.1	98.4	100
DOWN	Car_atr	0.0	0.0	2.7	21.3	13.3	46.7	0.0	0.0	0.0	1.3	4.0	5.3	34.7
DOWN	Pri_glu	2.8	2.8	23.1	40.6	33.3	1.3	0.5	0.0	0.0	16.5	75.0	55.1	21.7
DOWN	Cam_bar	9.3	84.0	96.0	98.7	92.0	39.3	14.8	15.3	52.5	98.6	95.5	83.4	43.9
DOWN	Leo_hi2	2.8	9.3	17.3	60.7	89.3	97.3	2.2	69.1	89.0	98.3	100	94.6	94.7
STABLE	Des_fle	44.4	40.0	53.3	36.2	37.8	17.9	0.0	-	-	-	-	-	-
STABLE	Cha_gla	0.0	6.7	35.6	53.3	44.4	24.4	0.4	0.0	0.0	22.1	23.2	0.0	0.0
STABLE	Thy_ser	15.6	37.8	24.4	25.1	35.6	13.3	11.0	58.5	54.2	22.2	47.0	28.0	23.7
STABLE	Vac_myr	0.0	0.0	5.3	87.3	73.6	45.3	0.0	5.3	71.7	93.3	60.8	76.2	1.3
STABLE	Hyp_ric	0.0	86.9	88.2	89.3	77.3	76.0	1.0	53.5	63.3	86.9	85.6	83.5	46.2
STABLE	Pla_ser	74.3	9.4	66.7	96.0	89.2	88.0	5.1	97.2	73.2	95.8	98.7	97.0	85.9
STABLE	Arm_can	34.3	98.7	94.7	98.7	88.0	87.0	1.1	42.1	97.3	98.7	93.2	86.1	69.9
STABLE	Pol_viv	71.1	75.6	66.7	51.1	40.0	8.9	7.8	14.4	50.5	41.9	77.9	59.3	51.1
STABLE	Car_rup	0.0	0.0	0.0	0.0	8.9	13.3	0.0	0.0	0.0	2.2	2.2	11.8	26.7
STABLE	Min_sed	21.1	1.3	4.0	20.2	41.3	40.0	0.7	40.0	56.0	65.7	73.3	75.8	48.7
STABLE	Ore_dis	4.0	18.9	13.2	29.3	14.3	0.0	1.1	1.3	13.6	34.7	31.4	29.3	5.6
STABLE	Geu_mo	12.0	4.0	2.7	33.3	26.7	30.7	15.9	92.5	82.3	93.7	86.2	88.5	67.8
STABLE	Car_cur	0.0	0.0	0.0	0.0	3.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
NEW	Log_arv	12.0	63.9	88.9	91.9	96.7	86.9	0.0	5.6	1.4	4.5	70.6	74.8	68.0
NEW	Rhi_ang	80.3	0.0	0.0	0.0	0.0	0.0	66.8	23.2	0.0	0.0	0.0	2.8	3.7
NEW	Ses_lib	5.3	29.3	66.7	63.8	50.7	8.0	47.2	94.7	93.3	92.7	96.0	85.2	58.7
NEW	Eup_sal	19.3	0.0	0.0	0.0	0.0	0.0	9.7	0.0	0.0	0.0	0.0	1.7	0.0
NEW	Sed_atr	0.0	4.0	0.0	5.3	13.3	6.8	0.0	1.3	1.3	0.0	5.5	0.0	0.0
NEW	Rho_fer	0.0	1.3	5.3	38.7	32.0	8.0	0.0	0.0	61.1	84.0	94.6	91.9	26.7
LOST	Res_com	0.0	1.3	2.7	42.7	62.2	74.0	0.0	0.0	0.0	21.6	77.8	93.2	90.9
LOST	Arm_ma	5.4	100	98.3	95.0	90.0	63.1	1.1	10.2	33.7	93.1	86.7	61.7	18.3
LOST	Phy_orb	0.0	8.0	0.0	0.0	0.0	0.0	1.3	17.1	24.7	16.6	17.5	12.3	9.7
LOST	Aco_lyc	42.2	0.0	0.0	0.0	0.0	0.0	8.8	87.0	56.1	16.7	3.3	3.0	8.7
LOST	Sol_vir	29.0	13.3	27.6	57.3	33.3	29.3	1.1	56.1	54.2	59.6	61.4	79.0	29.5
LOST	Rhi_gla	14.7	0.0	0.0	0.0	0.0	0.0	24.5	5.2	0.0	0.0	0.0	0.0	0.0

4. Ecological-related germination patterns in the genus *Saxifraga*

Abstract

The genus *Saxifraga* includes about 400 species with a predominantly mountain distribution. They show large morphological variations and adaptations to different habitats, representing an ideal context to assess ecological-related regenerative strategies in cold environments. Here I investigated germination patterns in 28 *Saxifraga* species from 6 different micro-habitats (rocky scree; dry rocky alpine grasslands; alpine grasslands; wet alpine grasslands/peatlands; stream bordures; tall herbs communities/woodland bordures) across the Alps.

Saxifraga seeds were exposed to four constant and two fluctuating temperatures, with and without cold stratification pre-treatments (i.e. 3 and 5 months of cold stratification), along with two different light treatments (i.e. 12/12 hour of light/darkness and full darkness), under controlled laboratory conditions. Multivariate analysis were used to group *Saxifraga* species sharing similar patterns of germination and dormancy, thereby defining three germination strategy clusters (Groups). Generalized Linear Models (GLM) were used to assess both single species and genus response to laboratory treatments. Subsequently Markov Chain – Monte Carlo Generalized Linear Mixed effect Models (MCMCGLMM) were used to assess if the germination and dormancy pattern differences among Groups can be explained by seed mass, plant ecology (expressed by Landolt ecological indicators) and species distribution ranges (arctic-alpine, alpine, south-european orophyte, euroasiatic), taking into account phylogenetic constrains.

Seed germination was promoted by decreasing incubation temperatures and by cold stratification in more than half of the species tested and it was strongly inhibited by darkness. Alpine, arctic-alpine and south-european orophyte species showed lower germination on average. Species with high germination at all conditions typically occur on nutrient-rich and humid soils (GroupA), while species with high germination only at low temperature or low germination in general, inhabit nutrient poor, dry basic (GroupB) and acidic soils (GroupC), respectively. Our results indicate that germination traits in the genus *Saxifraga* are overall unconstrained by phylogeny (except for light requirement), while they are driven by the species ecological niche (i.e. soil characteristics).

These results highlight that microenvironmental variables and species life-history play a major role than phylogeny and macroclimate (i.e. alpine) in determining the germination strategies across species.

Introduction

Saxifraga is the largest genus of the Saxifragaceae family, comprehending more than 450 species, of which 123 can be found in Europe. *Saxifraga* species are mainly distributed in alpine and temperate areas, with some in the circumpolar areas (Pan et al. 2001; Webb and Gornall 1989). In particular, 51 species (of which 21 endemic) occur on the European Alps. Most species are short-stature herbaceous plants, generally perennial, that reproduce primarily through small and light seeds (i.e. < 0.001g). The genus *Saxifraga* is ecologically diverse, being found in various microhabitats such as cliffs, screes, windy ridges, snowbeds and grasslands at high altitudes (Anchisi 1999; Aeschimann et al. 2004). This ecological breadth is reflected by morphological variations: for example, leaf area varies by at least a factor of 2500 among species (ranging $25cm^2$ to < $1mm^2$) and growth forms range from dense cushions to large un-branched rosettes and delicate broad-leaved herbs (Webb and Gornall 1989; Zhang 2013), providing some adaptations to local environmental conditions. Considering this ecological dissimilarity and yet, phylogenetic relatedness, *Saxifraga* species represent an ideal context to disentangle the role of these two primary drivers in defining seed dormancy and germination.

Seed germination is an irreversible process that must be timed to occur when the environment is favourable for subsequent seedling establishment, and it is primarily regulated by temperature, soil moisture and light (Poschlod et al. 2013; Fenner and Thompson 2005). Moreover, some species are completely inhibited to germinate until they have received an appropriate cue (Baskin and Baskin 2014), as germination can be mediated by seed dormancy. If a seed is dormant, certain environmental conditions are necessary to break its dormancy, allowing germination. Germination response to cold stratification periods is, for example, an adaptive trait that can be observed in many alpine species, which promote seedling emergence only after the winter has passed, avoiding germination in harsh condition (Billings and Mooney 1968; Baskin and Baskin 1998; Shimono and Kudo 2005). Responses to environmental cues have evolved into several different germination strategies and dormancy types that may reflect species adaptations to different habitats and climatic conditions (Willis et al. 2014). This is particularly evident in closely related species, where divergence in germination patterns could be explained by variation in micro-habitat, ecological breadth and geographic ranges (see Jiménez-Alfaro et al. 2016 and reference therein). Indeed, germination is extremely sensitive to environmental filters and play a crucial role in determining the distribution of adult stages in plant communities (Fraaije et al. 2015). This can result in germination trait similarities (i.e. trait convergence) among species within a given site (Larson and Funk 2016), and vice versa. Accordingly, recent studies have found close associations between germination strategies and dormancy with habitat/microhabitat preferences in several alpine species (Tudela-Isanta et al. 2018a, b).

Besides habitat characteristics, the physiological processes regulating seed dormancy and germination may depend on other factors, such as the phylogenetic position of the species (Nikolaeva 1999; 1977), their chorology (*sensu* Passalacqua 2015) (see Giménez-Benavides et al. 2005; Orsenigo et al. 2015; Tudela-Isanta et al. 2018a) and the seed mass (Grime et al. 1981). In this context, it is important to consider closely related species with similar geographic distributions and life cycles to study the correlation between seed germination requirements and habitats (Baskin and Baskin 2014). In fact, holding species life history relatively constant allow making habitat-related differences easier to detect (e.g Venable 1984, Meyer and Monsen 1991, and Carta et al. 2016). Moreover, different ecological condition experienced in the past have been found to be reflected in species germination ecology, as shown by arctic-alpine and south-european (S-EU hereafter) orophytes plants, which tend to be more dormant than the continental ones (Tudela-Isanta et al. 2018a). Conversely, there is not a consistent trend for the influence of seed mass on seed germination and dormancy, which can be either positively or negatively affected (Jurado and Flores 2005; Kahmen and Poschlod 2007; Norden et al. 2009).

Little is known about the germination ecology in the genus *Saxifraga*. Recent studies mainly focused on a single, or few *Saxifraga* species at time, investigating the germination response at only few temperatures, showing contrasting results. For example, *S. tridactylites* shows high levels of germination at low (5° - 10°C) temperatures (Pemadasa and Lovell 1975), while *S. italica*, was able to germinate only at warm temperature (20°C) (Di Cecco et al. 2019). Different germination response to temperature and cold stratification treatments have been observed both within and between several other species, as shown in *S. aizoides*, *S. oppositifolia*, *S. paniculata*, *S. hirculus*, *S. trifurcata* and *S. caniculata* (Ohlson 1989, Meier and Holdergger 1998, Cummins and Miller 2000, Schwienbacher and Erschbamer 2001, Giménez-Benavides and Milla 2013, Landi et al. 2016; Fernández et al. 2016). In some species seed germination was promoted by cold stratification (e.g. *S. paniculata*, *S. virginiensis*, *S. mutata*, *S. aizoides*, *S. oppositifolia*; Levin 1960; Holderegger 1996; Cummins and Miller 2000; Giménez-Benavides et al. 2005; Landi et al. 2016), while in some others it could occur also without it (e.g. *S. stellaris*, *S. hirculus*, *S. tridactylites*, *S. italica*; Pemadasa and Lovell 1975; Cummins and Miller 2000; Welch et al. 2002; Giménez-Benavides and Milla 2013; Di Cecco et al. 2019), indicating a different degree of dormancy in this genus. Unlike the responses to temperature and/or cold stratification, the germination responses to light seems to be more consistent across *Saxifraga* species. All the *Saxifraga* species resulted positively photoblastic, showing null or low germination in dark conditions, both in laboratory and field experiments (Bliss 1958; Levin 1960;

Meier and Holderegger 1998; Di Cecco et al. 2019). This is not surprisingly, since a positive germination response to light is a common trait of small seeded and ruderal plants (Thompson and Grime 1983; Poschlod 1991), as a mechanism to avoid germinating too deep in the soil, where seedling would deplete seed nutrients before reaching light for photosynthesis (Pons 2000; Flores et al. 2016). However, this trait was investigated in very few *Saxifraga* species (i.e. *S. aizoides*, *S. virginiensis*, *S. italica*, *S. punctata ssp. Nelsoniana*), thus knowledge about the genus germination response to light is lacking.

In this study, seeds of 28 species of *Saxifraga* of 7 Sections (Ligulatae, Porphyrion, Saxifraga, Trachyphyllum, Gymnopera, Cotylea and Michrantes), with different distribution (arctic-alpine, alpine, eurasiatic and S-EU orophytes) and inhabiting different alpine micro-habitats (i.e. rocky scree and rocks; dry rocky alpine grasslands; alpine grasslands; wet alpine grasslands and peatlands; stream bordures; tall herbs communities and woodland bordures), have been exposed to several laboratory conditions. In particular, seeds have been incubated at different temperatures (i.e., 5°C, 10°C, 15°C, 20°C, 25°C, 25/15°C and 15/5°C) and light conditions (12h/12h light/dark cycles and 24h darkness condition), with and without a cold stratification treatment (three or five months). I hypothesized that *Saxifraga* species have all photoblastic seeds, with different germination and dormancy patterns associated to species-specific ecological traits (approximated by Landolt ecological indicators F, R, Tl, N, D, H, L; Landolt 2010) and chorotype, while showing a weak phylogenetic constrain. In particular, I expected that species from wetter and nutrient-richer habitats show lower dormancy and wider temperature range for germination than species inhabiting nutrient-poorer and dryer habitats and that alpine and arctic-alpine species are characterized by deeper seed dormancy than eurasiatic species.

Material and Methods

Study species and seed collection

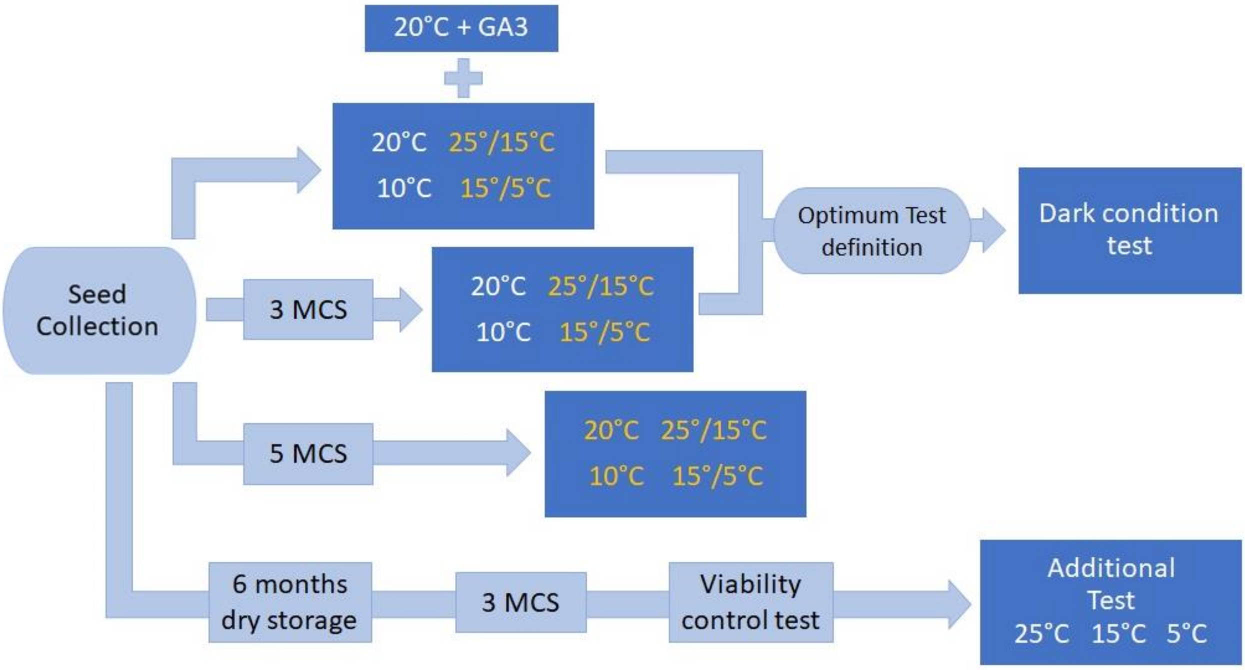
Seeds were collected from 28 species of *Saxifraga* at different locations all over the European Alps and Northern Apennine (Fig. S2) from the following alpine microhabitats: rocky scree and rocks; dry rocky alpine grasslands; alpine grasslands; wet alpine grasslands and peatlands; stream bordures; tall herbs communities and woodland bordures (defined according to Aeschimann, et al. 2004). For each species were also reported the respective Landolt indicator values (Landolt et al. 2010) and the chorology (*sensu* Passalacqua 2015) (Table 1).

Seeds collection was performed at time of their natural dispersal by the *Alpine Seed Conservation and Research Network* (Muller et al. 2017), in summer 2016 (21 species) and 2017 (7 species). For two species (*S. exarata* and *S. rotundifolia*), seeds of two different populations from different regions were also considered. After collection, seeds were stored at room temperature for approximately 2 months until the beginning of the experiments. Mean seed mass was obtained by weighting five replicate of 50 seeds each;

Germination tests

Experiments were conducted in temperature and light-controlled incubators (LMS 250A, LMS Ltd, Sevenoaks, UK), at 12-h daily photoperiod (PAR 40–50 µmol m−2 s−1). For each species and treatment, three samples of 25 seeds each were sown on 1 % distilled water-agar held in 50-mm-diameter Petri dishes. Considering the limited number of seeds available, the germination niche of each species has been defined following two main steps, designed to maximize the chances of germination testing. Firstly, I assessed whether all 28 *Saxifraga* species show different germination patterns at cool and warm conditions, incubating their seeds at two constant temperatures (10° and 20° C), with and without a period of three months of cold stratification pre-treatment (CS) at 0°C and full darkness (hereafter referred to as 0 MCS and 3 MCS, respectively). The 3 MCS was set up to investigate the presence of physiological dormancy across species. Furthermore, an additional germination test was set up at 20°C on 1% distilled water–agar containing gibberellic acid (GA_3, 722mM), to determine the level of physiological dormancy if present (Baskin and Baskin 2014, i.e. non-deep, intermediate, deep). Remnant seeds have been stored at dry room conditions to preserve their viability (15°C and 15%RH, ISTA 2019). Six months later, subsample of these seeds has been used to check the effect of dry storage on seed germination, by repeating one test at the best conditions previously found for each species. In the case of a not significant effect, the germination niche of each species have been further defined by exposing seeds to 3 MCS (since all species responded positively to CS) and then to two additional constant temperatures, either chosen among 5°, 15° or 25°C, depending on the best species-specific germination responses previously found. For example, if 10° and 20°C elicited both high final germination percentage in the initial tests, I subsequently tested 5° and 25°C; if final germination percentage was high at 20°C and low at 10°C, I tested 15° and 25°C, while if it was on the other way around, I tested 5° and 15°C). Furthermore, to evaluate the light requirements for germination, a third lot of seeds of each species was exposed to dark conditions (obtained by wrapping the plates in aluminium foil) at the test previously found to elicit the highest germination in light (hereafter referred as "optimum test") in that particular species. Additional experiments were performed to test the effect of daily alternating temperatures (i.e. 25°/15°, 15°/5°C, with light coinciding with the higher temperature and dark with the lower temperature) and long CS periods (5 months, hereafter 5 MCS) on seed germination. Due to seed shortage these latter tests could be performed only on 21 out of the 28 species (table S1).

Fig. 1 Germination tests summary. Germination tests are represented by blue boxes. Pre-treatments are represented by light blue boxes. Tests with incubation temperatures reported in orange were performed only on a subset of 21 species. MCS = Month of Cold Stratification.



Plates were checked for germination monthly during the cold stratification period and weekly (for 10 weeks) during the germination tests. Seeds were scored as germinated when the radicle protruded for a length greater than “seed diameter” (as *Saxifraga* seeds are very small, often < 1mm). 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) were calculated excluding non-viable seeds.

Statistical analysis

All calculations were performed by the means of the R environment for statistical computing (version 3.6.1, R Development Core Team 2019), and the software PAST (Hammer et al. 2015).

Seed germination across species

To investigate the effects of treatments and species identity on seed germination the Generalized Linear Models (GLM) with binomial distribution (probability ranging from 0 to 1), logit-link function and binomial error structure (or quasi-binomial error structure, if the residuals of the model were over-dispersed) (Venables and Ripley 1998) were used.

Firstly, a GLM was run with seed germination proportion (i.e., number of germinated seeds out of number of viable seeds of each species) as response variable, whereas temperature (i.e. 10 and 20°C), cold stratification period (i.e. 0 MCS and 3 MCS), species and their interactions were used as explanatory variables (fixed factors). Subsequently, for each species three additional GLM were performed to define the species germination response to the following fixed explanatory variables: (a) T, daily alternating/constant temperature (A/C, two-level categorical variable), CS (0, 3 and 5 MCS) and the interaction between CS and T; (b) light/darkness response (D/L, two-level categorical variable); and (c) response to phytohormones GA_3 (GA_3, two-level categorical variable). GLMs analysis were run in the R environment, with Package lmer (Bates et al. 2015). Significant differences among category levels were checked with the "Anova" function of Package "Car" (Fox and Weisberg 2019) and models assumptions where checked with the package "DHARMa" (Hartig 2019).

Dormancy types and classes of each *Saxifraga* species were defined following the Baskin and Baskin (2014) system of dormancy classification and Silveira's diagram (Silveira 2013), relying on embryo morphology data collected from the literature (Martin 1946) and the germination response obtained here. Additional information about the classification criteria used are reported in Table S2.

Multivariate Analysis

In order to define groups of species sharing recurring germination patterns, the FGP data for 10 and 20°C (after 0 MCS and 3 MCS) along with GA_3 and D/L test and seed weight, were analyzed with cluster analysis computed with Average linkage method (UPGMA with Euclidean distance) and k-means. Weight data were normalized prior to analyses to meet assumptions of normality and homogeneity of variance (See table 1). The appropriate number of species cluster was chosen considering the results of different combinations of number of clusters and clustering methods (taking in account cophenetic correlation coefficient values, Carvalho et al. 2019). Three germination strategy clusters (Groups hereafter) were identified and later compared and plotted using principal component analysis (PCA) using the "Facto-MineR" and "FactoExtra" packages (Kassambara and Mundt 2017; Lê et al. 2008). Significant differences among Groups were assessed by the means of PERMANOVA analysis and Tuckey HSD Post-Hoc test performed in the PAST environment.

Finally, non-parametric Kruskall-Wallis and Mann-Whitney tests were performed to identify possible statistical differences in terms of mean average Landolt ecological indicators' values within each Group (see Table 1). Analysis were carried out within the PAST environment.

Multiple species phylogenetic comparative analysis

A backbone phylogeny of the 28 S*axifraga* species was constructed based on *APGIV (Angiosperm Phylogeny Group 2016)* and on the phylogenetic tree realized by Tkach et al. (2018). Lower level nodes were subsequently resolved based on consensus molecular phylogenetic trees available. I analyzed how the germination varied at the genus level by fitting three Generalized Linear Mixed Models estimating posterior probability distribution for effects was estimated using a Markov chain Monte Carlo algorithm (Hadfield 2010) (MCMCGLMMs), with a binomial distribution to all the *Saxifraga* species germination proportion data. A first model (a) was fitted on a dataset including seed germination proportion data scored at 5°, 10°, 15°, 20° and 25°C with no or 3 MCS, with CS (binomial variable, 0=0 MCS, 1=3 MCS), T (continuous variable), "mass" (continuous variable), "Group" (binomial variable), "chorology" (binomial variable), "section" (binomial variable), "endemism" (binomial variable, 0=non-endemic species, 1= endemic species), and Landolt ecological indicators (seven continuous response variable, accounting for soil humidity (F), soil acidity (R), temperature (Tl), soil nutrient concentration (N), soil texture (D), light (L) and humus presence (H), Landolt et al. 2010), as fixed effect factors, along with their interactions terms. Secondly, two additional MCMCGLMMs, ran with the same fixed effect factors, were fitted on (b) a dataset including germination proportion data scored at the optimum T and MCS with 12h/12h light cycle (control) and in complete darkness condition, with "Darkness" (Binomial variable 0= darkness, 1= light) as an additional fixed effect factor (and its interaction terms), and (c) a dataset including germination proportion data scored at 10°, 15°/5°, 20° and 25°/15°C with 0 and 3 MCS, at 12h/12h light cycle, with "alternate T" (Binomial variable 0=constant T, 1= alternate T) as an additional fixed effect factor (and its interaction terms). The phylogenetic signal of all variables was calculated using Pagel's lambda (λ) (Pagel 1997, 1999).

Table 1 Chorology, section, endemicity, Landolt ecological indicators, dormancy type, seed mass, Group and microhabitat in which the species typically occur for each of the 30 taxa studied. Codes between brackets identify different populations of the same species. Microhabitats codes are a simplified version of those reported in Aeschimann *et al.* (2004): 1- rocky scree and rocks; 2- dry rocky alpine grasslands; 3- alpine grasslands; 4- wet alpine grasslands and peatlands; 5- stream bordures; 6- tall herbs communities and woodland bordures. Species that occurs only on siliceous or calcareous soils are indicated respectively with “a” or “b”, after the associated habitat code.

ID	Species	Chorology	Section	Endemism	F	R	Tl	N	D	H	L	Dormancy	Seed Mass (mg)	Group	Micro-habitat
1	*S. aizoides*	Art. Alp.	Porphyrion	-	4	4	2	2	2	1	4	n.d. PD	5.74	A	1, 4, 5
2	*S. androsacea*	Euras.	Saxifraga	-	3	4	1	1	3	1	4	ND	4.28	A	1, 4, 5
3	*S. aspera*	Oro. S-EU	Trachyphyllum	-	2	3	2	1	2	1	5	d. PD	1.38	C	1a, 2a
4	*S. bryoides**	Oro. S-EU	Trachyphyllum	-	2	2	2	1	2	1	5	i. PD	3.08	C	1a
5	*S. burseriana*	Alpine	Porphyrion	yes	2	4	2	1	1	1	4	n.d. PD	3.36	C	1b
6	*S. caesia*	Oro. S-EU	Porphyrion	-	2	4	2	3	2	2	3	n.d. PD	2.56	A	1, 3
7	*S. callosa*	Oro. S-EU	Ligulatae	-	2	4	3	1	1	1	4	ND	11.62	B	1b
8	*S. cochlearis*	Alpine	Ligulatae	yes	2	4	2	1	1	1	4	ND	8.16	B	1b
9	*S. cuneifolia*	Oro. S-EU	Gymnopera	-	3	2	2	4	3	4	2	n.d. PD	3.86	C	6
10a	*S. exarata* (lig)	Oro. S-EU	Saxifraga	-	2	2	1	1	2	1	5	ND	4.98	A	1, 3
10b	*S. exarata* (lom)	Oro. S-EU	Saxifraga	-	2	2	1	1	2	1	5	i. PD	3.56	C	1, 3
11	*S. moschata*	Euras.	Saxifraga	-	2	4	1	1	2	1	5	ND	3.66	A	1, 3
12	*S. facchinii**	Alpine	Saxifraga	yes	4	3	1	1	2	1	4	i. PD	7.44	C	1
13	*S. hostii*	Alpine	Ligulatae	yes	2	4	2	1	1	1	4	n.d. PD	4.76	C	1a
14	*S. blepharophylla**	Alpine	Porphyrion	yes	2	3	2	1	2	1	5	n.d. PD	6.46	C	1a
15	*S. oppositifolia**	Art. Alp.	Porphyrion	-	2	3	2	1	2	1	5	i. PD	4.40	C	1, 3
16	*S. paniculata*	Art. Alp.	Ligulatae	-	2	3	3	2	2	1	4	n.d. PD	6.24	C	1, 2
17a	*S. rotundifolia* (lig)	Oro. S-EU	Cotylea	-	4	3	1	4	3	3	2	ND	3.98	A	6
17b	*S. rotundifolia* (tos)	Oro. S-EU	Cotylea	-	4	3	1	4	3	3	2	ND	7.02	A	6
18	*S. seguieri**	Alpine	Saxifraga	yes	3	2	1	1	3	1	4	i. PD	2.66	C	1
19	*S. squarrosa*	Alpine	Porphyrion	yes	2	4	1	1	1	1	4	n.d. PD	2.44	A	1b
20	*S. stellaris*	Art. Alp.	Michrantes	-	4	3	2	1	2	1	4	ND	2.22	A	1, 5
21	*S. vandelli*	Alpine	Porphyrion	yes	2	4	1	1	1	1	4	n.d. PD	1.70	B	1b
22	*S. adscendens*	Art. Alp.	Saxifraga	-	2	3	2	2	2	2	4	i. PD	0.92	C	1, 2
23	*S. biflora*	Alpine	Porphyrion	yes	2	3	1	1	2	1	5	i. PD	3.88	C	1
24	*S. cotyledon*	Art. Alp.	Ligulatae	-	3	2	2	1	2	1	4	n.d. PD	2.60	C	1a
25	*S. mutata*	Oro. S-EU	Porphyrion	-	2	3	2	1	2	1	4	n.d. PD	1.58	A	1, 5
26	*S. rudolphiana*	Oro. S-EU	Porphyrion	-	4	3	1	3	4	2	4	ND	2.84	A	1
27	*S. sedoides*	Oro. S-EU	Saxifraga	-	3	3	2	1	3	1	4	d. PD	5.64	C	1
28	*S. tridactylites*	Euras.	Saxifraga	-	2	5	3	2	2	2	5	ND	0.55	B	1b, 2

When $\lambda = 0$, related taxa are not more similar than expected by chance (Pagel 1999), while when $\lambda = 1$, the trait is evolving following a constant variance random walk or Brownian motion model. λ was estimated simultaneously with the regression by calculating the mean of the posterior distribution and the 95% credible interval of λ as indicated by de Villemereuil and Nakagawa (2014). All analyses were run using the “MCMCglmm” package and the R environment (Hadfield 2010; R Core Team 2019) applying a non-informative prior, 60.000 MCMC iterations, burn-in after 10.000 iterations and a thinning interval of 50 iterations according to the documentation in Hadfield (2010). 5 MCS FGP data were excluded from these analysis, as long-term cold stratification treatments were not performed for all the species tested, and moreover, no statistically significant differences were found between 3 MCS and 5 MCS treatments for every Temperature tested, as confirmed by a GLMM fitted on 21 species seed germination proportion data.

Results

Saxifraga species germination and dormancy patterns

Seed germination response varied from 0 to 100%, with significant differences across species, incubation temperatures and stratification treatments (Table 2, Fig. S2). Because of the significant species × T and species × CS interactions (Table 2), species-specific analyses were performed (see Table S1). In particular, seed germination was positively related with increasing temperature in 2 species and negatively related in 9; FGP of *S. rotundifolia* showed an opposite response to temperature, depending on the populations tested. In the remaining 16 species, seed germination had no significant relation with T.

Seed germination increased significantly with increasing CS periods in 14 species, decreased only in one (*S. tridactylites*) and was not affect in 13. FGP of S. *rotundifolia* was either improved or not affected by CS, depending on the population tested. The effect of alternating temperature on FGP was not consistent across species, being either improved (*S. callosa*, and in both population of *S. rotundifolia*), constrained (*S. blepharophylla*, *S. paniculata*, *S. squarrosa*, *S. vandelli*) or showing no effect (other species), compared to daily constant regime. FGP of *S. exarata*, was either constrained or not affected by A/C, depending on the population tested (Table S1a). Germination response to GA_3 was consistently positive across species, while darkness had an inhibitory effect on seed germination in general (Table S1b-c). Following the system of dormancy classification shown in Table S2, 19 species resulted characterized by physiological dormancy (PD), while 8 species were non-dormant (ND). In the case of *S. exarata*, the two populations were either ND and i. PD. Among the dormant species, the non-deep level (n.d. PD) was the most abundant (11 of 20), followed by intermediate (i. PD) (7 of 20) and the remaining 2 species were classified as deep dormant (d. PD) (see Table 1).

Table 2 Results of the generalized linear model ANOVA of the effects of Species, Temperature (T), cold stratification (CS) and their interactions on seed germination.

Explanatory variables	χ^2	d.f.	*p*
Species	3237.5	29	**<0.001**
CS	463	29	**<0.001**
T	55.8	1	**<0.001**
Species × CS	644.2	29	**<0.001**
Species × T	940.2	29	**<0.001**
CS × T	3.8	1	n.s.
Species × CS × T	162.5	29	**<0.001**

Germination Strategy Cluster

The cluster analysis identified three germination strategy clusters (Group), which were significantly different, as attested by the PERMANOVA (Table S3, appendix). In the PCA performed on the germination percentage and seed weight, the total of variance explained by the two-principal axes was 76.3% (See Fig. 2). Axis I (representing 62.9% of the total variance) can be associated with increasing average FGP and the Axis II (representing 13.4% of the total variance) with germination response to temperature and seed mass (i.e., the higher the axis II value, the higher was the seed mass and the FGP at the lower temperature, and vice-versa, see Fig. S4). In accordance with the cluster analysis performed, the PCA displayed a different position of the three Groups and highlighted a higher within-Group variability for “GroupA” and “GroupB” than for “GroupC”.

Fig. 2 Principal component analysis (PCA) performed on the species germination. Species were clustered according to their germination patterns. Each dot represents a species. Confidence ellipses around the species clusters represent 0.95 C.I.

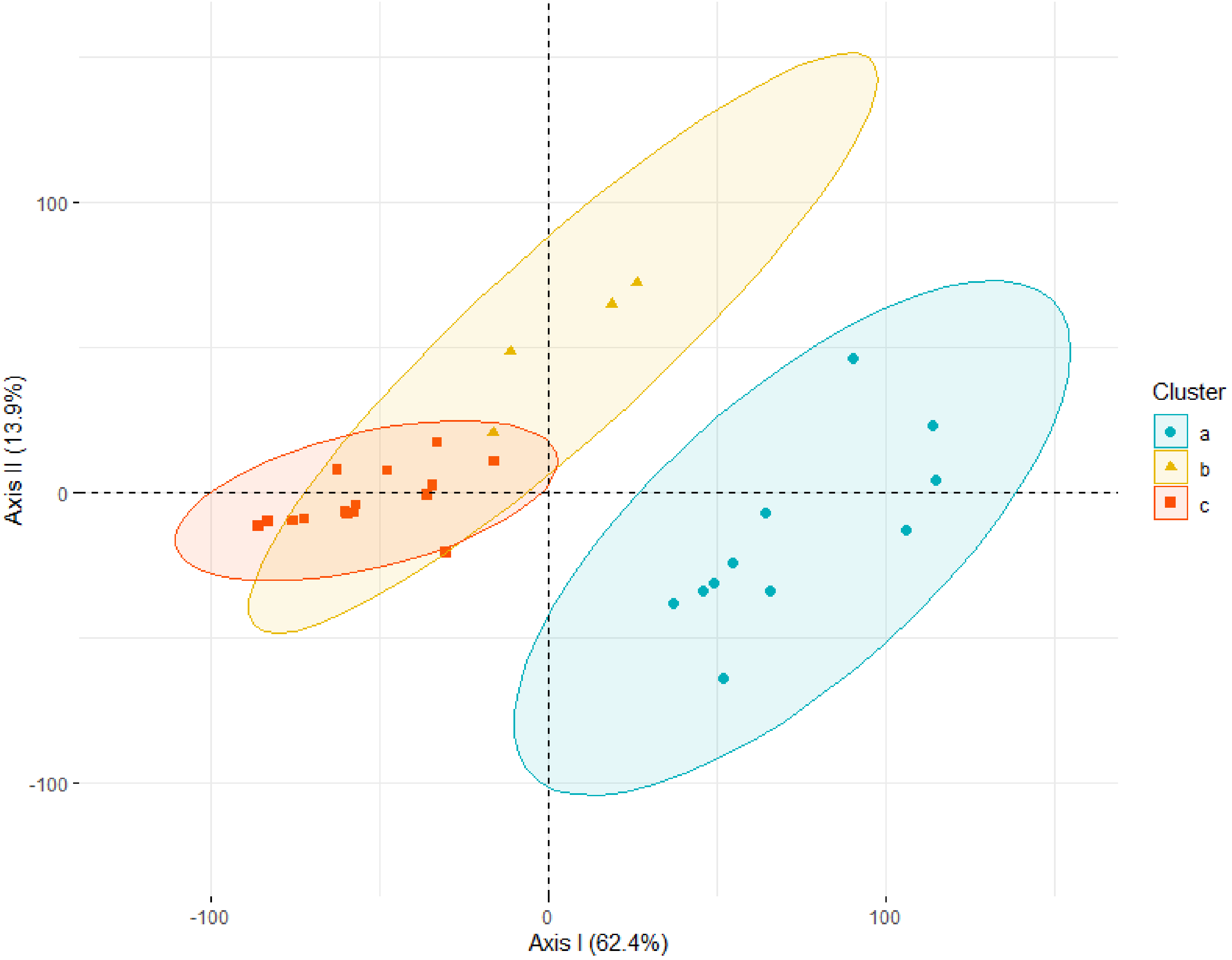
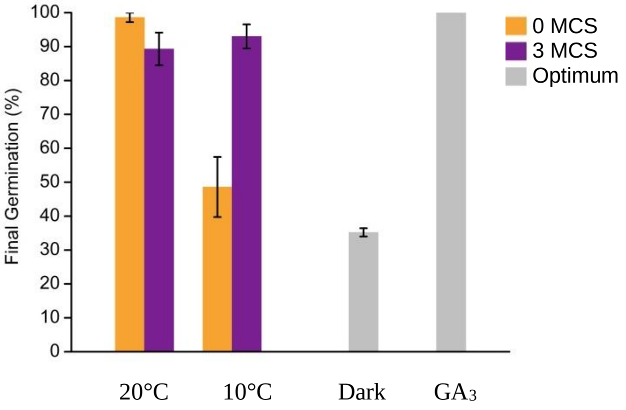
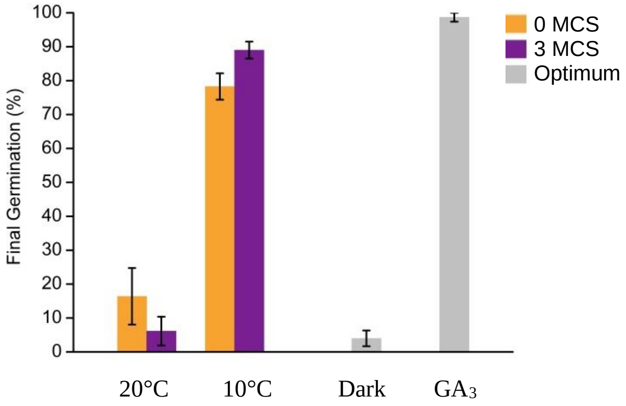
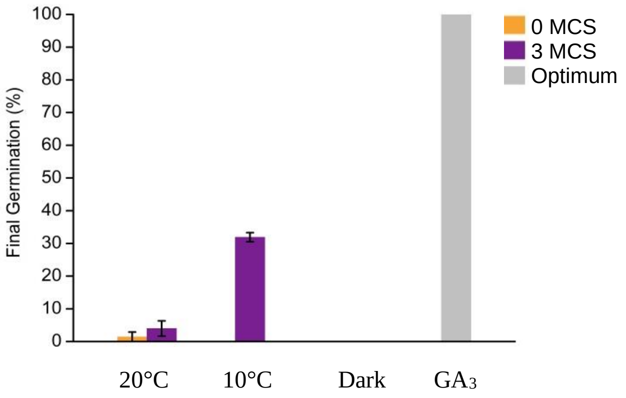
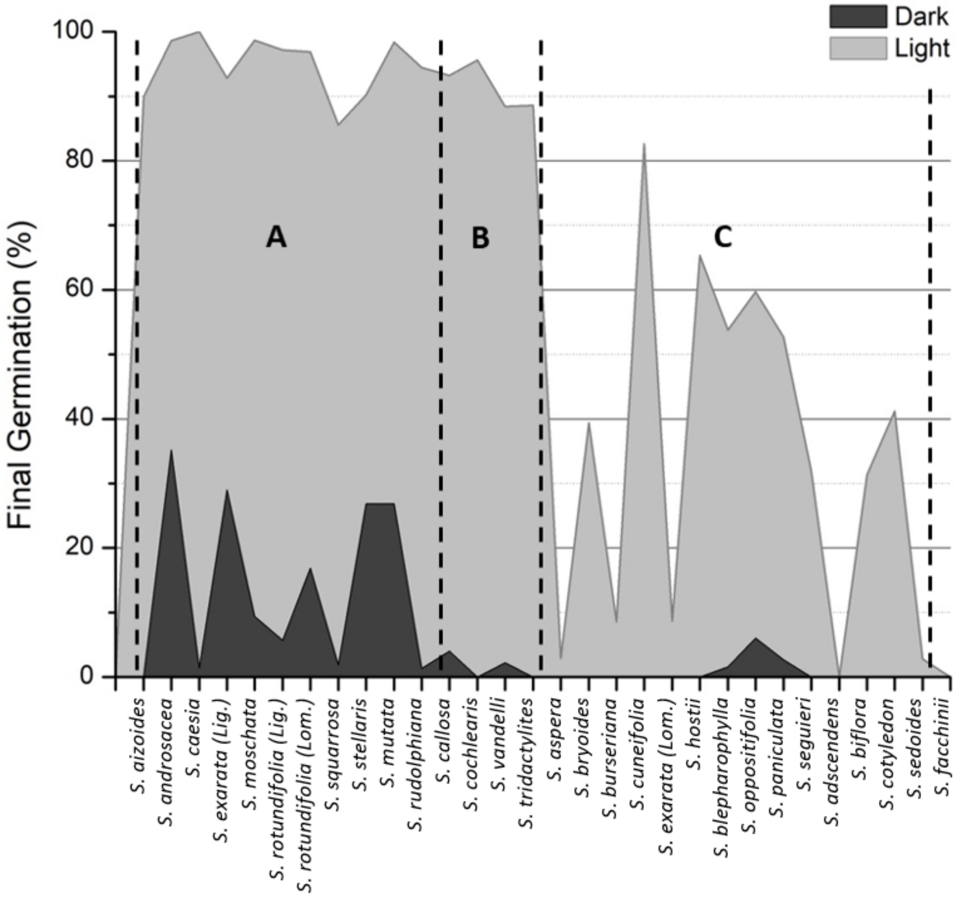


Table 3 Germination traits clusters (GroupA, GroupB and GroupC). For each cluster, a short description is provided, along with the list of species belonging to it. Codes between brackets identify different populations. The final germination percentage of each cluster is summarized using bar plots representing the results of one species (in bold) at 0 MCS (orange), after 3 MCS (purple), and under dark condition or with GA$_3$ at the optimal incubation T for that particular species (grey).

Germination Traits Cluster	Species	Germination response
GroupA) Includes ND or n.d. PD species, overall characterized by high FGP at both 10° and 20°C, with and without CS treatments. Few species showed germination (FGP always <40%) in dark condition.	*S. aizoides*, ***S. androsacea***, *S. caesia*, *S. exarata* (Lig.), *S. moschata*, *S. rotundifolia* (Lig.), *S. rotundifolia* (Tos.), *S. squarrosa*, *S. stellaris*, *S. mutata*, *S. oppositifolia* subsp. *rudolphiana*	
GroupB) Includes ND or n.d. PD species, characterized by high FGP only at 10°C. Very low germination was detected at dark condition (FGP<5%)	***S. callosa***, *S. cochlearis*, *S. tridactylites*, *S. vandellii*	
GroupC) Includes only dormant species, from non-deep to deep physiological dormancy, characterized by an overall low germination, if not at 10°C. Very low germination was detected at dark condition (FGP<7%)	*S. adsecendens*, *S. burseriana*, *S. cuneifolia*, *S. biflora*, ***S. seguieri***, *S. cotyledon*, *S. cotyledon*, *S. oppositifolia*, *S. oppositifolia* subsp. *blepharophylla*, *S. hostii*, *S. paniculata*, *S. aspera*, *S. sedoides*, *S. exarata* (Lom.), *S. facchinii*	

"GroupA" includes ND or n.d. PD species with high average FGP, and in particular, all species characterized by the highest FGP at high temperature and at darkness condition (i.e. 11 species); "GroupB" portrays species whose germination decreased at increasing T (i.e., 4 species), while "GroupC" (i.e. 15 species) includes the species with low and very low FGP, especially in absence of cold stratification treatment and at complete darkness condition (see also Fig. 3). A list of the species included in each Group is reported in Table 3, along with a summary of the germination traits shared and a bar plot depicting the typical FGP of two tests considered in the multivariate analysis. (i.e., FGP at 10° and 20° with 0MCS and 3MCS, along with GA_3 and Darkness tests).

Fig. 3 Final germination percentage of each species at their best incubation temperature at 12h/12h light/darkness (grey) and 24h darkness (black). Taxa are sorted by Groups.



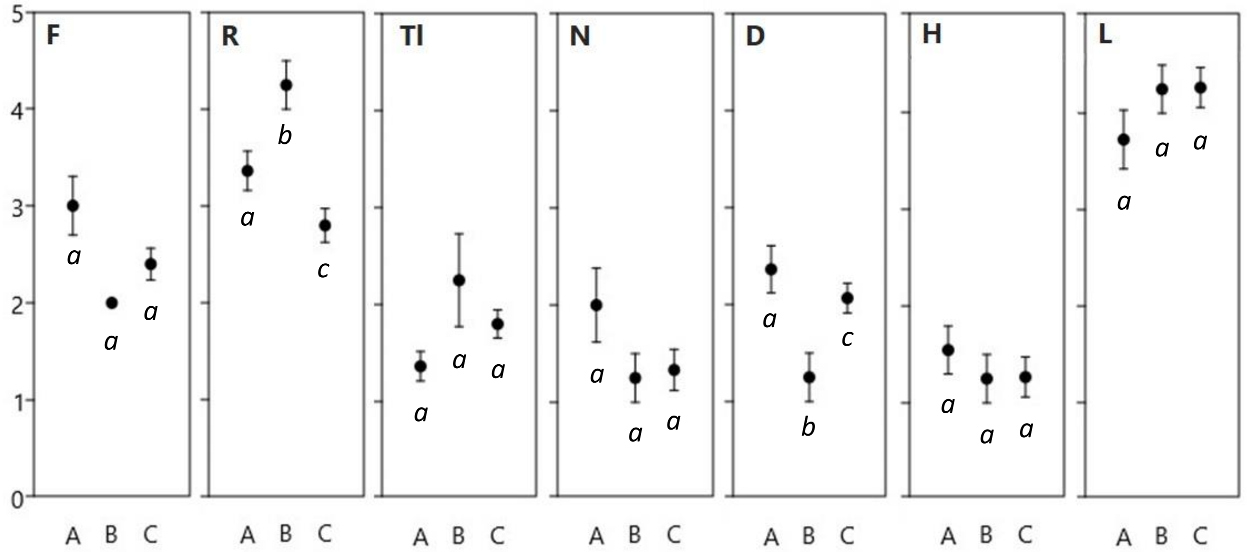
The three Groups defined by the Cluster analysis resulted characterized by species with different ecology, especially with significant differences for R (soil acidity) and D (soil texture) Landolt values (see Fig. 4). In particular, GroupA included species that are indicators of acidic/neutral soil (R) and richer in skeleton (D). Moreover, despite not statistically significant, the species of this Group show high F and N values, hence species that tend to occur in soils moderately wet and rich in nutrients.

Accordingly, GroupA includes species of rocky scree (e.g. *S. squarrosa*), alpine grasslands (from moderately dry to very wet, e.g. *S. moschata*, *S. androsacea*), streams borders (e.g. *S. stellaris* and *S. mutata*), humid and nitrophilous tall herbs communities (*S. rotundifolia*), thereby accounting for mainly species inhabiting wet or humid environments.

GroupB includes species that are indicators of basic (high R) and rocky soils, poor in skeleton (low D). Consequently, the species included in this Group are indicator of dry (low F) and nutrients-poor (low N) soils. In fact, GroupB comprehend species that can be found only on calcareous bare rocks (i.e. *S. callosa*, *S. cuneifolia* and *S. vandelli*), or very arid, calcareous and rocky grasslands (*S. tridactylites*). Similarly, GroupC includes species indicators of nutrient poor soils (low N), but with more skeleton (higher D). Moreover, despite not statistically significant, the species of this Group seem to prefer moderately dry (low F) and more acidic soils (lower R), compared to the other Groups. Species of GroupC grow almost exclusively on bare rock or scree, often siliceous (e.g. *S. blepharophylla*, *S. hostii*), with few species able to colonize also calcareous rocky habitat (e.g. *S. burseriana*) and non-rocky alpine grasslands (e.g. *S. exarata*, *S. oppositifolia*).

With regard to the Landolt indicators for humus abundance (H) and exposure to light (L), the species of the three Groups gave a rather homogeneous response, being all plants that usually grow on very humus-poor soils and in full light condition.

Fig. 4 Mean and Whisker plot for each Group (A, B, C) and Landolt ecological indicators (F = Moisture, R = Reaction/soil Ph., Tl = temperature, N = Nitrogen, D = Dispersity (soil texture), H = Humus, L = Light). Different letters indicate significant differences of Landolt indicator values across Groups at $p < 0.05$.



Phylogenetic signal and MCMCGLMM

At the *Saxifraga* genus level, seed germination showed a low phylogenetic signal, being Pagel's $\lambda < 0.600$ for the majority of the variable tested in the a)MCMCGLMM (moreover, with the 95% confidence intervals (C.I.) often overlapping with 0). FGP of the *Saxifraga* species is significantly inhibited by high temperature, as the majority of the species tested germinated better at cold condition. On the other hand, cold stratification treatment is a clear promoter for germination. The general response of germination to T and CS observed at a genus level resulted exacerbated for endemic species, which germinated more at cold incubation temperatures and after cold stratification, than the other *Saxifraga* species. Considering the Groups, the average germination of GroupA was higher than the null model, while the average germination of GroupB and GroupC was lower, even if not, or only marginally, significant. However, the effect of T and CS on germination varied greatly among the three Groups: species of GroupA showed a non-significant response to T (indicating the capacity of the species within this Group to germinate well at both high and low temperature), while seed germination was inhibited by high T in both GroupB and GroupC. Seed germination was also strongly promoted by cold stratification treatments in GroupA and GroupC, while, overall, germination in GroupB was not significantly affected by CS, as the majority of the species of this cluster are ND (see Table1). The low phylogenetic signal λ associated with the germination response to this Group (and its interactions with the other variables tested) suggests that the germination patterns observed cannot be linked to the species phylogenetic relation. Moreover, there were not significant differences of germination among the four chorotypes. However, looking at the interactions with T and CS, clear patterns can be observed: alpine, arctic-alpine and S-EU orophytic species are promoted by CS and cold T, while eurasiatic species are non-dormant and insensible to T. Interestingly, for the chorotype × T interaction, the phylogenetic signal λ was moderately high with CI not overlapping 0, suggesting that the germination response to T associated with chorotypes can be linked to a phylogenetic constrain among the species. No significant difference among germination response of different Sections (also considering its interaction with the other variables) and to seed weight were observed. Seed germination did not show any significant variation in response to the majority of the Landolt ecological indicators value, with the exception of soil acidity (R), which has a significant negative effect on germination. MCMCGLMM b) did not show any statistically significant difference in seed germination response to alternating or constant temperature (see Table 4b), also considering the possible interaction with the other variables included in the model. No phylogenetic signal either was detected ($\lambda < 0.600$, with CI overlapping 0). MCMCGLMM c) at the contrary highlights a very strong inhibiting effect of darkness condition on *Saxifraga* species germination. The effect of darkness was significant also in interaction with the other entire variable tested in the model, indicating a certain degree of variability among the species, even if the overall negative effect of darkness was anyway recognizable for each regression performed.

Table 4. Posterior mean estimates of the regression coefficient, *p*-values and λ (with 95% C.I. values in brackets) estimated during simple phylogenetic regressions based on the three MCMCGLMM performed, of the explanatory variables (with respective interaction) that resulted significant within the models.

Explanatory variables		**Posterior mean est.**	***p*-value**	**λ**
a)	**T**	-0.11	**<0.001**	0.417 (0.000; 0.876)
	CS	+1.41	**<0.001**	0.404 (0.000; 0.874)
	T × CS	+0.001	n.s.	0.404 (0.000; 0.868)
	Endemism × T	-0.143	**<0.001**	0.442 (0.000; 0.878)
	Endemism × CS	+1.760	**<0.01**	0.402 (0.000; 0.876)
	Group			
	-GroupA	+0.53	n.s.	0.509 (0.005; 0.849)
	-GroupB	-1.388	n.s.	0.509 (0.005; 0.849)
	-GroupC	-3.258	*0.06*	0.509 (0.005; 0.849)
	Group × T			
	-GroupA	+0.002	n.s.	0.487 (0.000; 0.810)
	-GroupB	-0.171	**<0.001**	0.487 (0.000; 0.810)
	-GroupC	-0.218	**<0.001**	0.487 (0.000; 0.810)
	Group × CS			
	-GroupA	+1.800	**<0.001**	0.390 (0.000; 0.859)
	-GroupB	+1.060	n.s.	0.390 (0.000; 0.859)
	-GroupC	+1.171	**<0.05**	0.390 (0.000; 0.859)
	Chorotype × T			
	-alpine	-0.180	**<0.001**	**0.709** (0.139; 0.971)
	-arctic-alpine	-0.132	**<0.01**	**0.709** (0.139; 0.971)
	-eurasiatic	+0.108	*0.051*	**0.709** (0.139; 0.971)
	-orophyte S-EU	-0.117	**<0.001**	**0.709** (0.139; 0.971)
	Chorotype × CS			
	-alpine	+2.018	**<0.001**	0.381 (0.000; 0.870)
	-arctic-alpine	+1.960	**<0.01**	0.381 (0.000; 0.870)
	-eurasiatic	-0.934	n.s.	0.381 (0.000; 0.870)
	-orophyte S-EU	+1.322	**<0.01**	0.381 (0.000; 0.870)
	Landolt (R)	+1.227	**<0.05**	0.047 (0.000; 0.896)
b)	**Alternate Temperature**	-0.36	n.s.	0.547 (0.000; 0.947)
c)	**Darkness**	-5.59	**<0.001**	**0.670** (0.322; 0.987)

The phylogenetic signal was high (not overlapping 0) for both the single variable Darkness and its interactions with the other variables included in the model (with $0.640 < \lambda < 0.799$, and with CI not overlapping 0), with the exception of the interaction with "seed mass". The rather high value of λ revealed the presence of phylogenetic signal in the *Saxifraga* seed germination response to darkness (see Table 4c and Fig. 3).

Discussion

Overall, the results presented here show that seed germination of alpine *Saxifraga* species is promoted by cold stratification treatment, inhibited by darkness and warm incubation temperatures and it is not affected by seed mass. The positive effect of cold stratification on seed germination is recognized a common trait in many alpine species, that is needed to break seed physiological dormancy, thereby promoting seedling emergence in spring (Densmore 1997; Baskin et al. 2002; Shimono and Kudo 2005; Baskin and Baskin 2014). Similarly, an obligate response to light for germination is a common trait in small-seeded species (Pons 2000; Fenner and Thompson 2005, Koutsovoulou et al. 2013), as those of some *Saxifraga* species (Bliss 1958; Levin 1960; Meier and Holderegger 1998; Di Cecco et al. 2019). Milberg et al. (2000) suggested that light response and seed mass coevolved as an adaptation to ensure germination of small-seeded species only when close to the soil surface, while Fenner and Thompson (2005) hypothesized a phylogenetic component for germination in light conditions, independently from of seed size. Accordingly, the results presented here show a significant phylogenetic influence for seed germination responses to light, though seed mass was similar across species. In addition to light, daily fluctuating temperatures are also a major factor in promoting the germination of small seeded species, which can fully or partially substitute for the light requirement (Baskin and Baskin 2014), working similarly as indicator of soil surface proximity (Probert 2000; Daws et al. 2002). Despite this, seed germination in the *Saxifraga* species tested here was not affected by fluctuating temperatures, supporting the view of Koutsovoulou et al. (2013) that the substitution of light by alternating temperatures for seed germination is a characteristic of light-promoted, yet larger, seeds.

Unlike the response to light, little is known about the temperature range for germination of small seeded-species, likely because current studies have used only few incubation temperatures per time. The results presented here show that most of the *Saxifraga* species tended to germinate better at cool incubation temperatures (i.e. 5°-15°C), highlighting novel insights about the germination requirement of small-photoblastic seeds, at least from alpine environment. This result contrasts with early and recent studies on germination ecology of several alpine species that show either high temperature requirements (Sayers and Ward 1966; Chabot and Billings 1972; Marchand and Roach 1980; Kibe and Mazusawa 1994; Cavieres and Arroyo 2000) or highly heterogeneous responses, respectively (see Walk et al. 2011 and reference therein). The requirement of high temperatures for germination has been considered an adaptation of alpine species to prevent early spring or autumn germination when a high likelihood of frost may result in a low probability of seedling establishment (Cavieres and Arroyo 2000). On the other hand, the high variety of germination responses found later in alpine species have been linked to large species and microhabitats diversity of these environments (Hoyle et al. 2015; Körner 2003; Schwienbacher et al. 2011; Tudela-Isanta et al. 2018a, b). In fact, frost damage is not the only cause of high seedling mortality in alpine species, which is also affected by heat stress in summer (Marcante et al. 2014). Topsoil desiccation and hyperthermia in summer are common in some alpine environments (Jumpponen et al. 1999, Gieger and Leuschner 2004), especially in bare ground and rocky substrates, poor in skeleton and in exposed locations (Neuner et al. 1999; Kammer and Möhl 2016), where most of the *Saxifraga* species thrive (Anchisi 1999, Aeschimann et al. 2004). Desiccation risk may be exacerbated for seedlings of *Saxifraga* species, given their obligate photoblastic germination response (see above) that constrain emergence only at the soil surface. Consequently, *Saxifraga* species would germinate mostly early in spring, soon after snow melt, when water is available and temperature are cool enough to prevent drought. In addition, earlier seedling establishment may result in better development of the root system before summer, which help overcoming the hazards of the hot season (Peñaloza 1996, Cavieres and Arroyo 2000).

Beyond these general patterns, our results highlight different species-specific germination strategies within the genus *Saxifraga*, involving different degree of dormancy and optimum temperatures for germination. These strategies could be clustered into three main Groups characterized each by species sharing similar microhabitats, thereby underlining ecological-related germination traits. In accordance with my hypothesis, species usually found in microhabitats with high water, nitrogen and soil nutrients availability (i.e. GroupA, Fig. 3), showed lower restrictions for seed germination (e.g. several ND species, wide temperature range for germination), compared to species of the other two Groups, indicating that conditions for successful seedling recruitment may occur also right after dispersal, such as late summer or autumn. Supporting this view, recent studies have shown that autumn-emerged seedlings of alpine species could survive across the winter (Marcante et al. 2012; Mondoni et al. 2015). Autumn germination may be even advantageous in the short growing season of the alpine environments, ensuring seedlings are well placed to grow quickly when temperatures begin to rise in early spring (Tudela-Isanta et al. 2018a). In the strategy summarized for “GroupB” the majority of the species are characterized by n.d. PD, showing high FGP only at cool temperatures (e.g. ≤ 15°C Table 1b). The species from this Group occur on dry/very dry and calcareous rocky soils poor in nutrients, in which soil desiccation and hyperthermia are common in summer (Kammer and Möhl 2016, see above). For these reasons, the strong inhibitory effect of high temperature (e.g. ≥20°C) on germination could be interpreted as an adaptation to prevent seed germination when the risk of drought is high. The fact that a few species can germinate also without CS (except *S. vandelli*, which is dormant), suggests that late summer or autumn germination is possible also for *Saxifraga* species inhabiting dry-calcareous soils, provided that temperature are cool enough (e.g. < 15°C) and water is available. Unlike many annual alpine species that are characterized by dormant seeds (Hájková and Krekule 1972), with a persistent soil seed bank (Ohtsuka and Ohsawa 1994), the winter annual *S. tridactylites* shows high FGP (e.g. > 85%) only in fresh seeds and at cool temperatures (e.g. 10°C). Cold stratification induced dormancy in the seeds of this species, suggesting that its primary strategy to germinate is immediately after dispersal, in late summer or autumn (see above). These results indicate that non-germinated seeds enter in a deep state of dormancy and build soil seed bank after winter periods. This difference from the other *Saxifraga* species can be explained by the broad ecological niche of this eurasiatic plant: *S. tridactylites* is in fact, not exclusive of the alpine and montane altitudinal belt, as it can be found in disturbed rocky and arid habitat along an altitudinal gradient ranging from 0 to 2500 m a.s.l. (Anchisi 1999).

The majority of *Saxifraga* species from siliceous bedrock of acidic rocky cliffs or screes (i.e. from GroupC) were characterized by i. PD or d. PD being able to germinate only at cool condition (e.g. ≤15°C) after CS treatments (Table 1c). The germination strategy observed here suggest that these species would mostly germinate in spring, soon after snow melt, when water is available and temperatures are still cool, thereby avoiding both summer drought and early autumn/spring frost episodes (Graae et al. 2009; Marcante et al. 2012; Marcante et al. 2014). Unlike species from previous Groups, species from GroupC were not able to germinate in absence of CS. Species from siliceous habitats needs higher water potential to germinate than species from calcareous, basic soils (Tudela-Isanta et al. 2018b); thus, a germination strategy that promote the recruitment of these species in periods of the year when water availability is high is not surprising. Moreover, the significant relation between R Landolt's indicator values for pH and germination (i.e. lower germination in siliceous, acidic soil), suggests that soil acidity act as a main driver for seed germination (see also Tudela-Isanta et al. 2018a, b; Fernández-Pascual et al. 2017). However, here I have found that the role of pH was weaken under wet conditions (i.e. GroupA accounts for species from both basic and acidic soils, that reached high FGP at different conditions), indicating that this trait is particularly important only in dry soils.

The phylogenetic signal λ associated with the seed germination response to T and CS, along with that observed for the strategy patterns portrayed by Group, were fairly low (with CI values that overlap 0). This suggests that germination patterns observed here are not explained by a phylogenetic relationship among the species (i.e. species more closely related do not behave more similar than others); consequently, the origin of these patterns should be attributed to different microhabitat characteristics. Accordingly, temperature constrains for germination and dormancy were found not phylogenetically affected also in other alpine species (of different genus, see Fernández-Pasqual et al. 2017; Tudela-Isanta et al. 2018a).

Germination differences among chorotypes tested in this study highlighted that, similarly to what has been observed in Tudela-Isanta et al. (2018a), arctic–alpine, alpine, and S-EU orophytes germination was more positively promoted by cool temperature and cold stratification, than eurasiatic species. Interestingly, this effect appeared co-evolved within non-euroasiatic species, as the associated phylogenetic signal for this trait was high. Consequently, germination traits in the genus *Saxifraga* seems influenced by the species distribution. This difference supports the hypothesis that seed dormancy is partly affected 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 acted in preventing germination after dispersal, which would decrease the probability of encountering subsequent unfavourable growth conditions (Probert 2000). On the other hand, no phylogenetic signal was observed among species from different chorotypes in response to cold stratification. Accordingly, dormancy patterns were found to be independent from phylogenetic constrains in several plant families (e.g. Fabaceae, Asteraceae and Euphorbiaceae, see Jurado and Flores 2005), while they more associated with environmental variability.

4.7 Concluding remarks

Seed germination in the genus *Saxifraga* resulted promoted by cold stratification and cool incubation temperatures, while it was strongly inhibited by darkness. These traits suggest that *Saxifraga* species tends to germinate at the soils surface, immediately after snow melts in early spring, when water availability is high and temperatures are cool enough to prevent topsoil desiccation. Consequently, besides being photoblastic, small seeded *Saxifraga* species require low temperature for germination; the possibility that these germination patterns occur in other small-seeded species cannot be ruled out. Beyond these common traits, it was possible to observe different germination and dormancy patterns across species, which could be clustered into three main strategies. These strategies reflected the species ecological niche, especially the requirements for water availability, soil acidity and texture. On the other hand, there was no phylogenetic influence in the germination response of *Saxifraga* species, except for light requirements (also associated to the small seed size) and chorotype.

Consequently, here I highlight that microenvironmental variables and species life-history play a major role than phylogeny and macroclimate (i.e. alpine) in determining the germination strategies across species.

4.8 References

- Aeschimann, D., Lauber, K., and André Michel, D. (2004). *Flora alpina: atlante delle 4500 piante vascolari delle Alpi*. Zanichelli.
- Anchisi, E. (1999). Sassifraghe delle Alpi e degli Appennini.
- Baskin, C.C., Zackrisson, O. and Baskin, J.M. (2002) Role of warm stratification in promoting germination of seeds of Empetrum hermaphroditum (Empetraceae), a circumboreal species with a stony endocarp. American Journal of Botany 89, 486–493.
- Baskin, C.C. and Baskin, J.M. 2014. Seeds: ecology, biogeography, and evolution of dormancy and germination. Academic Press, New York, NY, US.
- Bates, D., Maechler, M., Bolker, B., and Walker, S. (2015). lme4: Linear mixed-effects models using Eigen and S4. R package version 1.1–7. 2014.
- Billings, W. D., and Mooney, H. A. (1968). The ecology of arctic and alpine plants. Biological reviews, 43(4), 481-529.
- Bliss, L. C. (1958). Seed germination in arctic and alpine species. Arctic, 11(3), 180-188.
- Carta, A., Hanson, S., and Müller, J. V. (2016). Plant regeneration from seeds responds to phylogenetic relatedness and local adaptation in Mediterranean Romulea (Iridaceae) species. Ecology and Evolution, 6(12), 4166-4178.
- Cavieres, L. A., and Arroyo, M. T. (2000). Seed germination response to cold stratification period and thermal regime in Phacelia secunda (Hydrophyllaceae)–altitudinal variation in the Mediterranean Andes of central Chile. Plant Ecology, 149(1), 1-8.
- Chabot, B. F., and Billings, W. D. (1972). Origins and ecology of the Sierran alpine flora and vegetation. Ecological Monographs, 42(2), 163-199.
- Cummins, R. P., and Miller, G. R. (2000). The role of chilling in the germination of some Scottish montane species. Botanical Journal of Scotland, 52(2), 171-185.
- Daws, M. I., Burslem, D. F. R. P., Crabtree, L. M., Kirkman, P., Mullins, C. E., and Dalling, J. W. (2002). Differences in seed germination responses may promote coexistence of four sympatric Piper species. Functional Ecology, 16(2), 258-267.
- Densmore, R. V. (1997). Effect of day length on germination of seeds collected in Alaska. American Journal of Botany, 84(2), 274-278.
- Di Cecco, V., Di Musciano, M., Frattaroli, A. R., and Di Martino, L. (2019). Seed ecology of Saxifraga italica: effects of light, temperature and gibberellic acid. Folia Geobotanica, 1-12.
- Fenner, M. K., and Thompson, K. (2005). The ecology of seeds. Cambridge University Press.

- Fernández-Pascual, E., Jiménez-Alfaro, B., and Bueno, Á. (2017). Comparative seed germination traits in alpine and subalpine grasslands: higher elevations are associated with warmer germination temperatures. Plant Biology, 19(1), 32-40.
- Flores, J., González-Salvatierra, C., and Jurado, E. (2016). Effect of light on seed germination and seedling shape of succulent species from Mexico. Journal of Plant Ecology, 9(2), 174-179.
- Fox, J., and Weisberg, S. (2019). Using car Functions in Other Functions.
- Fraaije, R. G., ter Braak, C. J., Verduyn, B., Breeman, L. B., Verhoeven, J. T., and Soons, M. B. (2015). Early plant recruitment stages set the template for the development of vegetation patterns along a hydrological gradient. Functional Ecology, 29(7), 971-980.

- Fernández-Pascual, E., Jiménez-Alfaro, B., and Bueno, Á. (2017). Comparative seed germination traits in alpine and subalpine grasslands: higher elevations are associated with warmer germination temperatures. Plant Biology, 19(1), 32-40.

- Gieger, T., and Leuschner, C. (2004). Altitudinal change in needle water relations of Pinus canariensis and possible evidence of a drought-induced alpine timberline on Mt. Teide, Tenerife. Flora-Morphology, Distribution, Functional Ecology of Plants, 199(2), 100-109.
- Giménez-Benavides, L., Escudero, A., and Pérez-García, F. (2005). Seed germination of high mountain Mediterranean species: altitudinal, interpopulation and interannual variability. Ecological Research, 20(4), 433-444.
- Giménez-Benavides, L., and Milla, R. (2013). Comparative germination ecology of two altitudinal vicariant Saxifraga species endemic to the north of Spain. Plant Biology, 15(3), 593-600.
- Grime, J. P., Mason, G., Curtis, A. V., Rodman, J., and Band, S. R. (1981). A comparative study of germination characteristics in a local flora. The Journal of Ecology, 1017-1059.
- Graae, B. J., Ejrnæs, R., Marchand, F. L., Milbau, A., Shevtsova, A., Beyens, L., and Nijs, I. (2009). The effect of an early-season short-term heat pulse on plant recruitment in the Arctic. Polar Biology, 32(8), 1117.
- Hadfield, J. D. (2010). MCMC methods for multi-response generalized linear mixed models: the MCMCglmm R package. Journal of Statistical Software, 33(2), 1-22.
- Hájková, L., and Krekule, J. (1972). The developmental pattern in a group of therophytes: I. Seed dormancy. Flora, 161(1-2), 111-120.
- Hammer, Ø. (2015). PAST: Paleontological Statistics. Reference Manual. Natural History Museum, University of Oslo. Oslo, Norway.

- Hartig, F (2019). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.2.4.
- Hoyle, G. L., Steadman, K. J., Good, R. B., McIntosh, E. J., Galea, L. M., and Nicotra, A. B. (2015). Seed germination strategies: an evolutionary trajectory independent of vegetative functional traits. Frontiers in plant science, 6, 731.
- Jiménez-Alfaro, B., Silveira, F. A., Fidelis, A., Poschlod, P., and Commander, L. E. (2016). Seed germination traits can contribute better to plant community ecology. Journal of Vegetation Science, 27(3), 637-645.
- Jumpponen, A., Väre, H., Mattson, K. G., Ohtonen, R., and Trappe, J. M. (1999). Characterization of 'safe sites' for pioneers in primary succession on recently deglaciated terrain. Journal of Ecology, 87(1), 98-105.
- Jurado, E., and Flores, J. (2005). Is seed dormancy under environmental control or bound to plant traits?. Journal of Vegetation Science, 16(5), 559-564.
- Kahmen, S., and Poschlod, P. (2007). Does germination success differ with respect to seed mass and germination season? Experimental testing of plant functional trait responses to grassland management. Annals of botany, 101(4), 541-548.
- Kammer, P. M., and Möhl, A. (2002). Factors controlling species richness in alpine plant communities: an assessment of the importance of stress and disturbance. Arctic, Antarctic, and Alpine Research, 34(4), 398-407.
- Kassambara, A., and Mundt, F. (2017). Package 'factoextra'. Extract and visualize the results of multivariate data analyses, 76.
- Kibe, T., and Masuzawa, T. (1994). Seed germination and seedling growth of Carex doenitzii growing on alpine zone of Mt. Fuji. Journal of Plant Research, 107(1), 23-27.
- Körner, C. (1999). Alpine plants: stressed or adapted. Physiological Plant Ecology. Blackwell, Oxford, 297-312.
- Körner, C. (2003). Alpine plant life: functional plant ecology of high mountain ecosystems; with 47 tables. Springer Science and Business Media.
- Koutsovoulou, K., Daws, M. I., and Thanos, C. A. (2013). Campanulaceae: a family with small seeds that require light for germination. Annals of Botany, 113(1), 135-143.
- Landi, M., Biondini, S., Zoccola, A., Gobbo, G., Betti, A., Saveri, C., ... and Angiolini, C. (2016). Effect of cold treatment on germination of Saxifraga aizoides and S. paniculata: a comparison of central (eastern Alps) and southern populations (northern Apennines). Biologia, 71(2), 149-154.

- Landolt, E., Bäumler, B., Ehrhardt, A., Hegg, O., Klötzli, F., Lämmler, W., ... and Urmi, E. (2010). Flora indicativa: Okologische Zeigerwerte und biologische Kennzeichen zur Flora der Schweiz und der Alpen. Haupt.
- Larson, J. E., and Funk, J. L. (2016). Regeneration: an overlooked aspect of trait-based plant community assembly models. Journal of Ecology, 104(5), 1284-1298.
- Lê, S., Josse, J., and Husson, F. (2008). FactoMineR: an R package for multivariate analysis. Journal of statistical software, 25(1), 1-18.
- Levin, M. H. (1960). Studies on the ecological life history of Saxifraga virginiensis. Bulletin of the Torrey Botanical Club, 348-354.
- Marcante, S., Sierra-Almeida, A., Spindelböck, J. P., Erschbamer, B., and Neuner, G. (2012). Frost as a limiting factor for recruitment and establishment of early development stages in an alpine glacier foreland?. Journal of Vegetation Science, 23(5), 858-868.
- Marcante, S., Erschbamer, B., Buchner, O., and Neuner, G. (2014). Heat tolerance of early developmental stages of glacier foreland species in the growth chamber and in the field. Plant ecology, 215(7), 747-758.
- Marchand, P. J., and Roach, D. A. (1980). Reproductive strategies of pioneering alpine species: seed production, dispersal, and germination. Arctic and Alpine research, 12(2), 137-146.
- Martin, A. C. (1946). The comparative internal morphology of seeds. The American Midland Naturalist, 36(3), 513-660.
- Meier, C., and Holderegger, R. (1998). Breeding system, germination, and phenotypic differences among populations of Saxifraga aizoides (Saxifragaceae) at the periphery of its alpine distribution. Nordic Journal of Botany, 18(6), 681-688.
- Meyer, S. E., and Monsen, S. B. (1991). Habitat-correlated variation in mountain big sagebrush (Artemisia tridentata ssp. vaseyana) seed germination patterns. Ecology, 72(2), 739-742.
- Milberg, P., Andersson, L., and Thompson, K. (2000). Large-seeded spices are less dependent on light for germination than small-seeded ones. Seed science research, 10(1), 99-104.
- Mondoni, A., Pedrini, S., Bernareggi, G., Rossi, G., Abeli, T., Probert, R. J., ... and Orsenigo, S. (2015). Climate warming could increase recruitment success in glacier foreland plants. Annals of Botany, 116(6), 907-916.
- Müller, J. V., Berg, C., Détraz-Méroz, J., Erschbamer, B., Fort, N., Lambelet-Haueter, C., ... and Porro, F. (2017). The Alpine Seed Conservation and Research Network-a new initiative

to conserve valuable plant species in the European Alps. Journal of Mountain Science, 14(4), 806-810.

- Neuner, G., Ambach, D., and Aichner, K. (1999). Impact of snow cover on photoinhibition and winter desiccation in evergreen Rhododendron ferrugineum leaves during subalpine winter. Tree Physiology, 19(11), 725-732.
- Nikolaeva, M. G. (1977). Factors controlling the seed dormancy pattern. Physiology and Biochemistry of Seed dormancy and Germination.
- Nikolaeva, M. G. (1999). Patterns of seed dormancy and germination as related to plant phylogeny and ecological and geographical conditions of their habitats. Russian Journal of Plant Physiology, 46, 369-373.
- Norden, N., Daws, M. I., Antoine, C., Gonzalez, M. A., Garwood, N. C., and Chave, J. (2009). The relationship between seed mass and mean time to germination for 1037 tree species across five tropical forests. Functional Ecology, 23(1), 203-210.
- Ohlson, M. (1989). Ecotypic differentiation and phenotypic plasticity in Saxifraga hirculus populations in central and northern Sweden. Ecography, 12(1), 46-53.
- Ohtsuka, T., and Ohsawa, M. (1994). Accumulation of buried seeds and establishment of ruderal therophytic communities in disturbed habitat, central Japan. Vegetatio, 110(1), 83-96.
- Orsenigo, S., Abeli, T., Rossi, G. et al (2015) Effects of autumn and spring heat waves on seed germination of high mountain plants. PLOS One 10(7): e0133626 doi:10.1371/journal.pone.0133626
- Pagel, M. (1997). Inferring evolutionary processes from phylogenies. Zoologica Scripta, 26(4), 331-348.
- Pagel, M. (1999). Inferring the historical patterns of biological evolution. Nature, 401(6756), 877.
- Pan, J.-T., Gornall, R.J., and Ohba, H. 2001. Saxifraga L. *In* Flora of China. Vol. 8. *Edited by* Z.-Y. Wu, and P.H. Raven. Science Press; Missouri Botanical Garden Press., Beijing; St. Louis. pp. 280–345.
- Passalacqua, N. G. (2015). On the definition of element, chorotype and component in biogeography. Journal of Biogeography, 42(4), 611-618.
- Pemadasa, M. A., and Lovell, P. H. (1975). Factors controlling germination of some dune annuals. The Journal of Ecology, 41-59.
- Peñaloza, A. (1996). Consideraciones microclimáticas en la sobrevivencia de semillas plántulas de Kageneckia angustifolia D. Don: una especie del bosque esclerófilo montano de

Chile central (Doctoral dissertation, Tesis de Magíster, Facultad de Ciencias, Universidad de Chile, Santiago, Chile).

- Pons, T. L. (2000). Seed responses to light. Seeds: the ecology of regeneration in plant communities, 2, 237-260.
- Poschlod, P. (1991). Diasporenbanken in Böden—Grundlagen und Bedeutung. In Populationsbiologie der Pflanzen (pp. 15-35). Birkhäuser, Basel.
- Poschlod, P., Abedi, M., Bartelheimer, M., Drobnik, J., Rosbakh, S., and Saatkamp, A. (2013). Seed ecology and assembly rules in plant communities. Vegetation ecology, 164-202.
- Probert, R. J. (2000). The role of temperature in the regulation of seed dormancy and germination. Seeds: the ecology of regeneration in plant communities, 2, 261-292.
- R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
- Sayers, R. L., and Ward, R. T. (1966). Germination responses in alpine species. Botanical Gazette, 127(1), 11-16.
- Schaal, B. A., and Leverich, W. J. (1981). The demographic consequences of two-stage life cycles: survivorship and the time of reproduction. The American Naturalist, 118(1), 135-138.
- Schwienbacher, E., and Erschbamer, B. (2002). Longevity of seeds in a glacier foreland of the Central Alps–a burial experiment. Bulletin of the Geobotanical Institute ETH, 68, 63-71.
- Schwienbacher, E., Navarro-Cano, J. A., Neuner, G., and Erschbamer, B. (2011). Seed dormancy in alpine species. Flora-Morphology, Distribution, Functional Ecology of Plants, 206(10), 845-856.
- Shimono, Y., and Kudo, G. (2005). Comparisons of germination traits of alpine plants between fellfield and snowbed habitats. Ecological Research, 20(2), 189-197.
- Silveira, F. A. O. (2013). Sowing seeds for the future: the need for establishing protocols for the study of seed dormancy. Acta Botanica Brasilica, 27(2), 264-269.
- Thompson, K., and Grime, J. P. (1983). A comparative study of germination responses to diurnally-fluctuating temperatures. Journal of applied Ecology, 141-156.
- Tkach, N., Röser, M., Miehe, G., Muellner-Riehl, A. N., Ebersbach, J., Favre, A., and Hoffmann, M. H. (2015). Molecular phylogenetics, morphology and a revised classification of the complex genus Saxifraga (Saxifragaceae). Taxon, 64(6), 1159-1187.
- Tudela-Isanta, M., Fernández-Pascual, E., Wijayasinghe, M., Orsenigo, S., Rossi, G., Pritchard, H. W., and Mondoni, A. (2018a). Habitat-related seed germination traits in alpine habitats. Ecology and evolution, 8(1), 150-161.

- Tudela-Isanta, M., Ladouceur, E., Wijayasinghe, M., Pritchard, H. W., and Mondoni, A. (2018b). The seed germination niche limits the distribution of some plant species in calcareous or siliceous alpine bedrocks. Alpine Botany, 128(1), 83-95.
- Venable, D. L. (1984). Using intraspecific variation to study the ecological significance and evolution of plant life-histories. Perspective on Plant Population Ecology, 166-187.
- Venables, W. N., and Ripley, B. D. (1998). Modern Applied Statistics with S-Plus,(3rd corrected printing).
- de Villemereuil, P., and Nakagawa, S. (2014). General quantitative genetic methods for comparative biology. In Modern phylogenetic comparative methods and their application in evolutionary biology (pp. 287-303). Springer, Berlin, Heidelberg.
- Webb, D. A., and Gornall, R. J. (1989). *Saxifrages of Europe* (pp. 307-307). London: Christopher Helm.
- Welch, D. (2002). The establishment of recovery sites for Saxifraga hirculus L. in NE Scotland. Botanical Journal of Scotland, 54(1), 75-88.
- Willis, C. G., Baskin, C. C., Baskin, J. M., Auld, J. R., Venable, D. L., Cavender-Bares, J., ... and NESCent Germination Working Group. (2014). The evolution of seed dormancy: environmental cues, evolutionary hubs, and diversification of the seed plants. New Phytologist, 203(1), 300-309.
- Zhang, Z. (2013). Phylogenetic studies in the genus Saxifraga (Saxifragaceae) (Doctoral dissertation, University of Leicester).

Appendix

Table S1. Results of the GLM for the germination response of each species to: a) Temperature (T), daily alternating temperature (A/C), cold stratification (CS) and its interaction with T; b) Complete darkness conditions; c) Phytohormones (GA_3). Significant *p*-values are highlighted in bold. Marginally significant *p*-values are highlighted in italic. Alternate temperature Test were not performed for species from 22 to 28. Additional constant Temperature test were not performed for species highlighted by asterisks.

ID	**Species**	**a)**								**b)**		**c)**	
		T		**A/C**		**CS**		**CS × T**		**Darkness**		**GA_3**	
		Estim.	*p*-value	Estim.	*p*-value	Estim.	*p*-value	Estim.	*p*-value	Estim.	*p*-value	Estim.	*p*-value
1	*S. aizoides*	+0.01	n.s.	-0.20	n.s.	+ 1.14	**<0.01**	-0.27	n.s.	-6.31	**<0.001**	+6.94	**<0.001**
2	*S. androsacea*	+0.46	**<0.05**	+0.38	n.s.	+0.91	n.s.	-0.04	n.s.	-4.83	**<0.001**	+0.75	n.s.
3	*S. aspera*	-0.11	n.s.	-0.99	n.s.	+0.18	n.s.	+0.01	n.s.	-0.69	n.s.	+1.53	n.s.
4	*S. bryoides**	-0.17	**<0.01**	-0.39	*0.05*	+0.08	n.s.	+0.02	*0.05*	-3.78	**<0.001**	+2.41	**<0.05**
5	*S. burseriana*	-0.17	***0.07***	-0.06	n.s.	+0.61	n.s.	-0.01	n.s.	-1.25	n.s.	+4.24	**<0.001**
6	*S. caesia*	+0.20	n.s.	-1.81	n.s.	+5.08	*0.08*	-0.18	n.s.	-8.55	**<0.001**	+2.00	**<0.001**
7	*S. callosa*	-0.29	**<0.001**	+0.81	**<0.01**	+0.71	**<0.05**	-0.03	**<0.05**	-5.80	**<0.001**	+4.47	**<0.001**
8	*S. cochlearis*	-0.33	**<0.001**	+0.60	n.s.	+0.83	**<0.05**	-0.03	n.s.	-7.35	**<0.001**	+5.74	**<0.001**
9	*S. cuneifolia*	-0.11	n.s.	+0.50	n.s.	+0.23	n.s.	-0.01	n.s.	-2.86	**<0.001**	+6.70	**<0.001**
10a	*S. exarata* (lig)	-0.23	**<0.001**	-1.70	**<0.001**	+0.27	n.s.	-0.02	n.s.	-3.46	**<0.001**	+3.99	**<0.001**
10b	*S. exarata* (lom)	-0.24	**<0.05**	+0.80	n.s.	-0.48	n.s.	+0.02	n.s.	-1.98	n.s.	+3.41	**<0.01**
11	*S. moschata*	+0.11	n.s.	+0.15	n.s.	+0.36	n.s.	-0.01	n.s.	-6.54	**<0.05**	+1.41	**<0.05**
12	*S. facchinii**	-4.79	n.s.	-20.48	n.s.	-9.54	n.s.	+0.96	n.s.	-0.08	n.s.	+3.11	**<0.01**
13	*S. hostii*	-0.16	n.s.	-0.82	n.s.	+1.57	**<0.001**	-0.06	**<0.05**	-4.93	**<0.001**	+4.04	**<0.001**
14	*S. blepharophylla**	-0.10	n.s.	-0.65	<**0.05**	+0.69	**<0.01**	-0.01	n.s.	-4.20	**<0.001**	+3.30	**<0.001**
15	*S. oppositifolia*	-0.17	*0.07*	-0.06	n.s.	+0.74	**<0.05**	-0.02	n.s.	-3.12	**<0.001**	+0.00	n.s.
16	*S. paniculata*	-0.11	n.s.	-0.93	**<0.01**	+1.38	**<0.01**	-0.06	**<0.05**	-3-67	**<0.001**	+3.90	**<0.001**
17a	*S. rotundifolia* (lig)	+0.13	**<0.01**	+2.57	**<0.001**	+1.13	**<0.001**	-0.04	**<0.01**	-6.35	**<0.001**	+2.15	**<0.05**
17b	*S. rotundifolia* (tos)	-0.12	**<0.05**	+1.04	**<0.001**	-0.02	n.s.	+0.01	n.s.	-5.05	**<0.001**	+1.92	n.s.
18	*S. seguieri**	-0.17	**<0.05**	-0.42	n.s.	+0.8	**<0.01**	-0.01	n.s.	-3.46	**<0.001**	+8.43	**<0.001**
19	*S. squarrosa*	+0.09	n.s.	-1.02	**<0.05**	+1.84	**<0.001**	-0.08	**<0.05**	-3.70	**<0.001**	+5.33	**<0.001**
20	*S. stellaris*	+0.13	*0.06*	-0.29	n.s.	+0.09	n.s.	-0.01	n.s	-3.20	**<0.001**	+4.38	**<0.001**
21	*S. vandelli*	-0.36	**<0.001**	-1.12	**<0.001**	+0.85	**<0.01**	+0.01	n.s.	-5.81	**<0.001**	+4.79	**<0.001**
22	*S. adscendens*	-0.01	n.s.	-	-	-24.7	n.s.	+1.29	n.s.	-0.39	n.s.	+5.57	**<0.001**
23	*S. biflora*	-0.05	n.s.	-	-	-0.21	n.s.	0.04	n.s.	-3.27	**<0.01**	+6.29	**<0.001**
24	*S. cotyledon*	-0.30	**<0.01**	-	-	+0.11	n.s.	0.03	n.s.	-3.88	**<0.001**	+6.68	**<0.001**
25	*S. mutata*	-0.06	n.s.	-	-	+2.34	**<0.001**	-0.05	n.s.	-5.13	**<0.001**	+7.56	**<0.001**
26	*S. rudolphiana*	-0.10	n.s.	-	-	-0.67	n.s.	+0.03	n.s.	-6.87	**<0.001**	+4.44	**<0.001**
27	*S. sedoides*	+0.01	n.s.	-	-	+1.81	**<0.01**	-0.18	**<0.01**	-0.74	n.s.	+0.75	n.s.
28	*S. tridactylites*	-0.30	**<0.001**	-	-	-3.42	**<0.001**	+0.10	**<0.01**	-6.50	**<0.001**	+2.45	**<0.001**

Table S2 Dichotomous key to the 2 class and levels of dormancy of the 28 *Saxifraga* species interfered from germination tests and literature (i.e. seed coat permeability and embryo type)

1. FGP without cold stratification treatment was ≥70% at any temperature
1.1 Yes .... **Non-Dormant**
1.2 No **2**
2. Is seed coat permeable?
2.1 Seed coat is not permeable.. **PY**
2.2 Seed coat is permeable.. **3**
3. Is embryo fully developed when dispersed?
3.1 Embryo is undifferentiated or underdeveloped when dispersed.. **4**
3.2 Embryo is fully developed when dispersed **6**
4. Is FGP without cold stratification treatment ≥70% at any temperature?
4.1 Yes..**MD**
4.2 No.. **5**
5. Embryo grows during cold stratification (3 or 5 months)
5.1 GA_3 substituted for cold stratification in promoting germination .... **MPD-I-complex**
5.2 GA_3 did not substitute for cold stratification in promoting germination..**MPD-D-complex**
5.3 Gibberellic acid or cold stratification does not promote germination..**unknown-MPD**
6. Did GA_3 promote germination without cold stratification treatment ?
6.1 Yes **7**
6.2 No **8**
7. Did short cold stratification treatment promoted germination at any temperature ?
7.1 Yes .... **Non-deep PD**
7.2 No .... **Intermediate PD**
8. Did long cold stratification treatment promoted germination at any temperature ?
8.1 Yes, but germination was observed also after short stratification treatment .... **Intermediate PD**
8.2 Yes, but no germination was observed after short stratification treatment .... **Deep PD**
8.3 FGP was low in all conditions tested .... **Deep PD**

Fig. S1 (part 1) Mean FGP (with standard error) for the 30 taxa tested in laboratory. MCS0 = fresh seeds; MCS3 = seed stratified for 3 months; MCS5 = seed stratified for 5 months. Only 3MCS FGP are available for the "additional germination test" (either two of 5°, 15° or 25°C; see material and methods for details). Additional germination tests not performed are highlighted by "X"

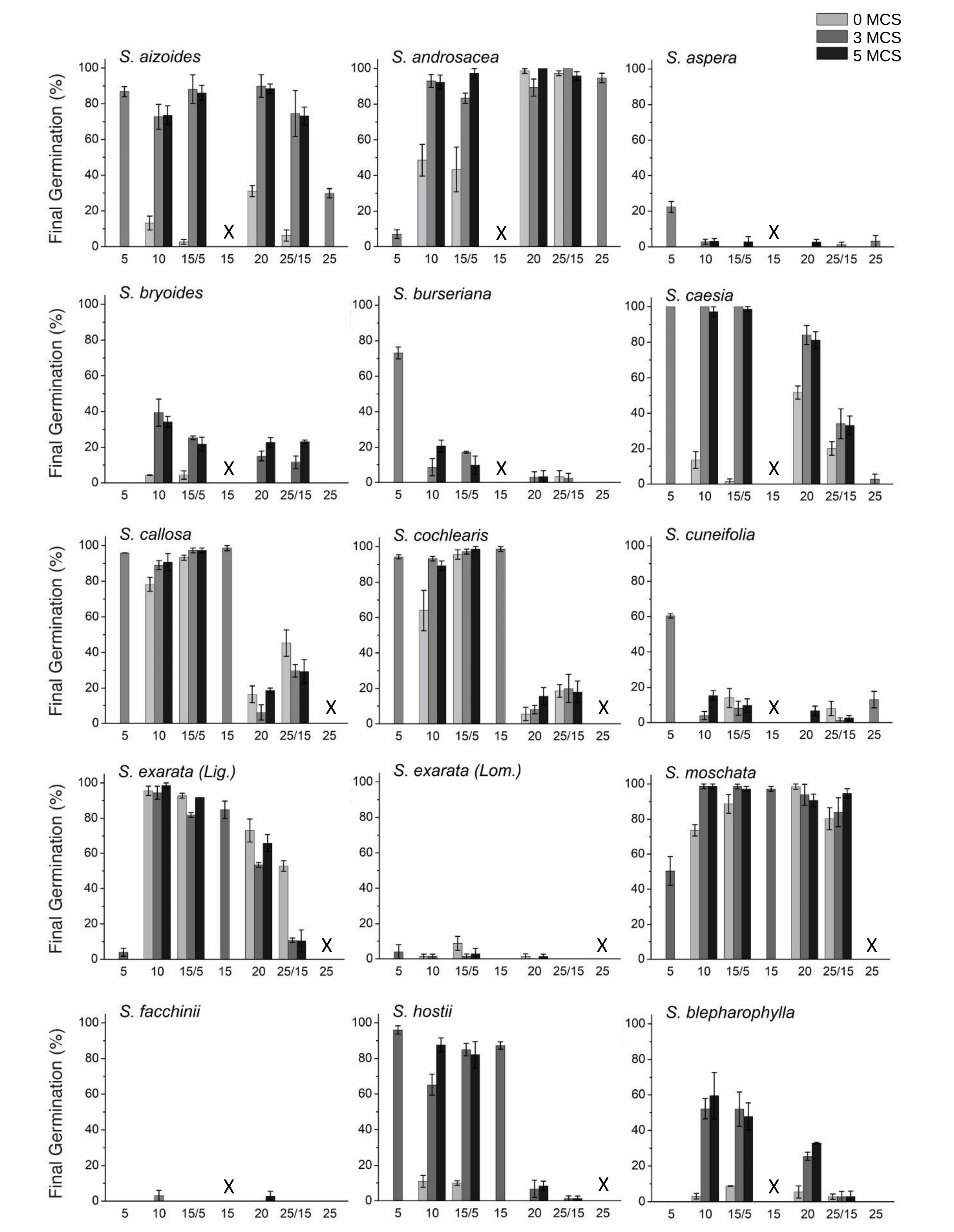


Fig. S1 (part 2) Mean FGP (with standard error) values for the 30 taxa tested in laboratory. MCS0 = fresh seeds; MCS3 = seed stratified for 3 months; MCS5 = seed stratified for 5 months. Only 3MCS FGP are available for the "additional germination test" (either two of 5°, 15° or 25°C; see material and methods for details). Additional germination tests not performed are highlighted by "X"

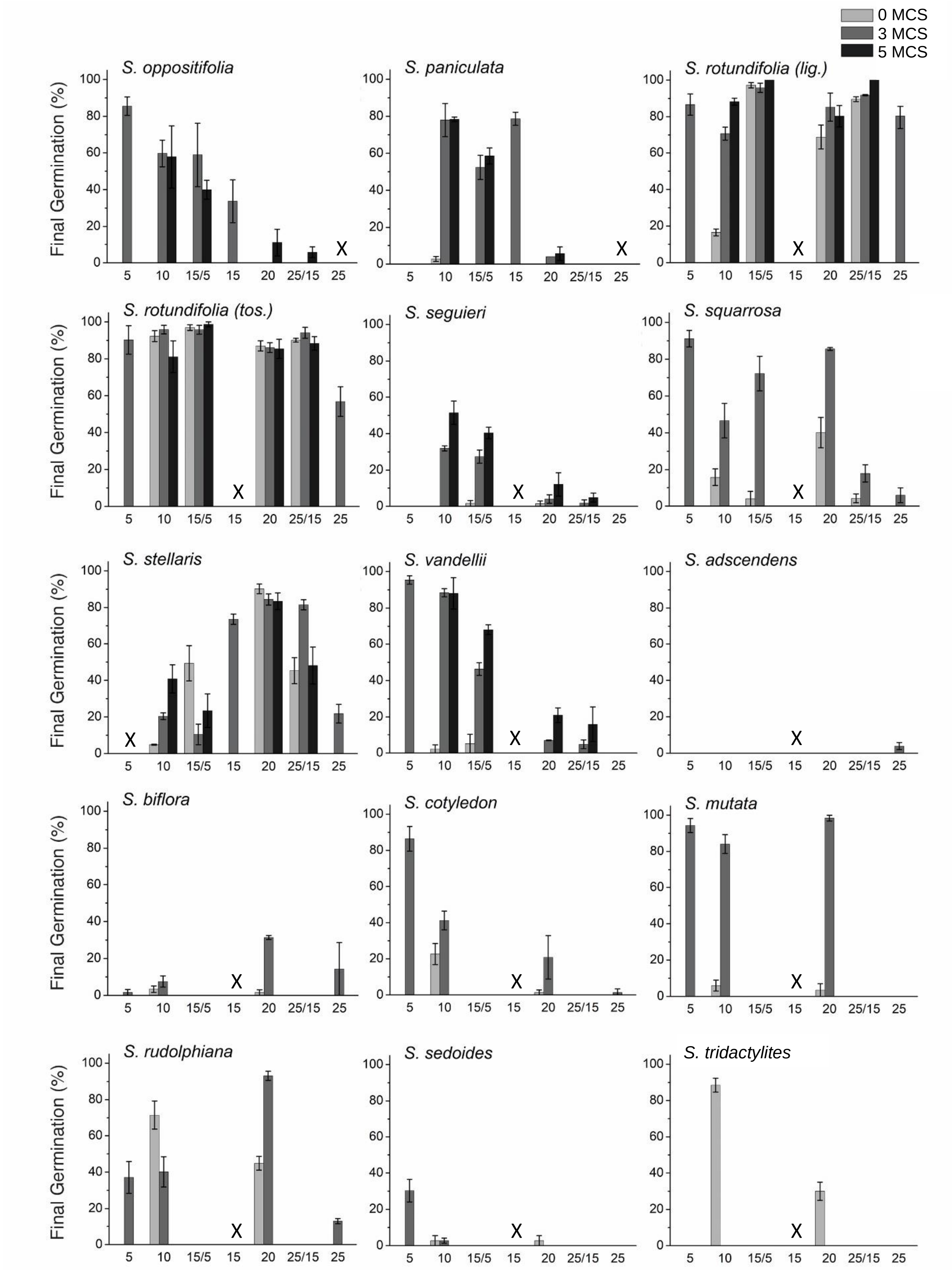


Fig. S2 *Saxifraga* seed collection sites.

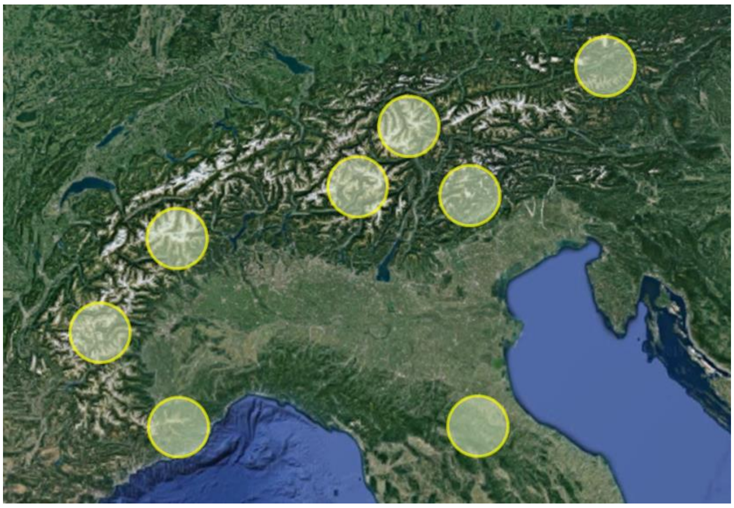


Fig. S3 *Saxifraga* species Phylogenetic tree

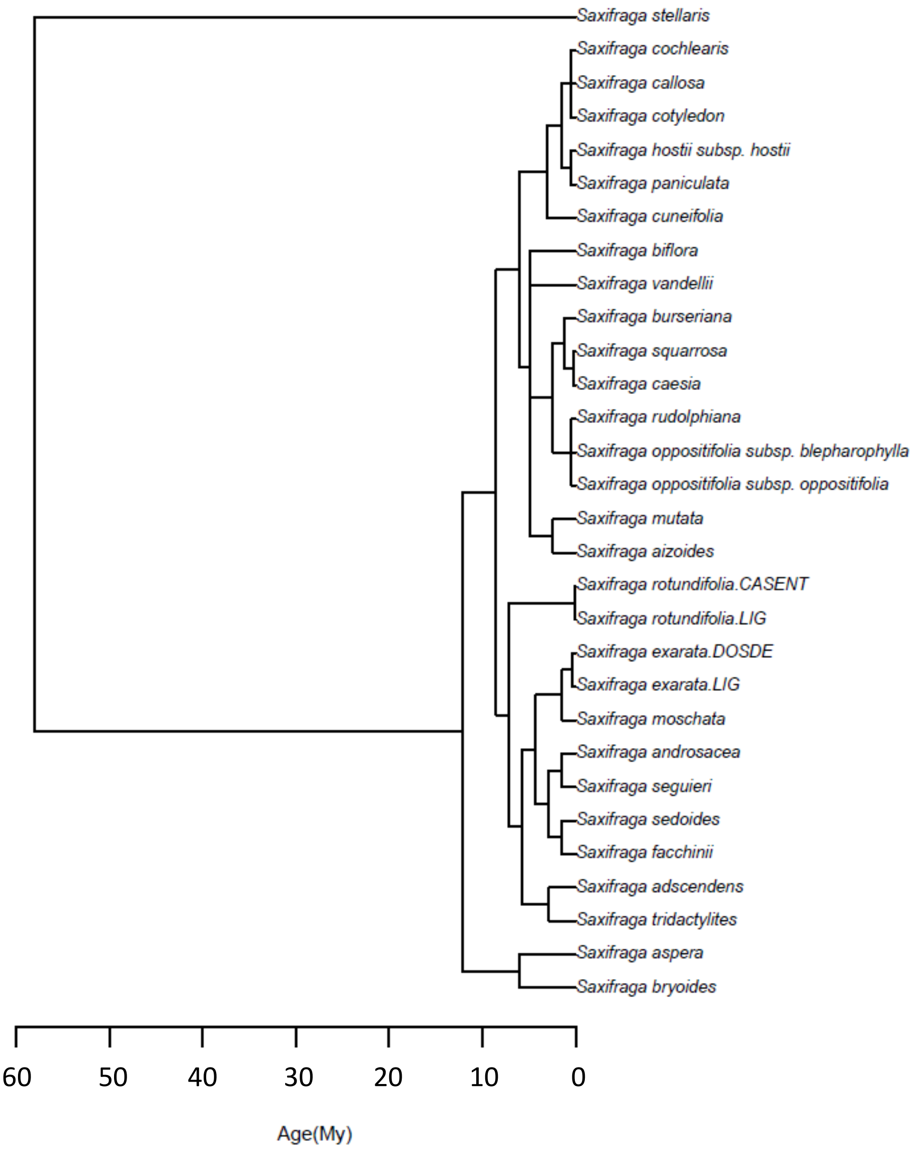


Fig. S2 UPGMA Clustering of *Saxifraga* species (distance measure used: Euclidean). Cophenetic correlation coefficient = 0.823

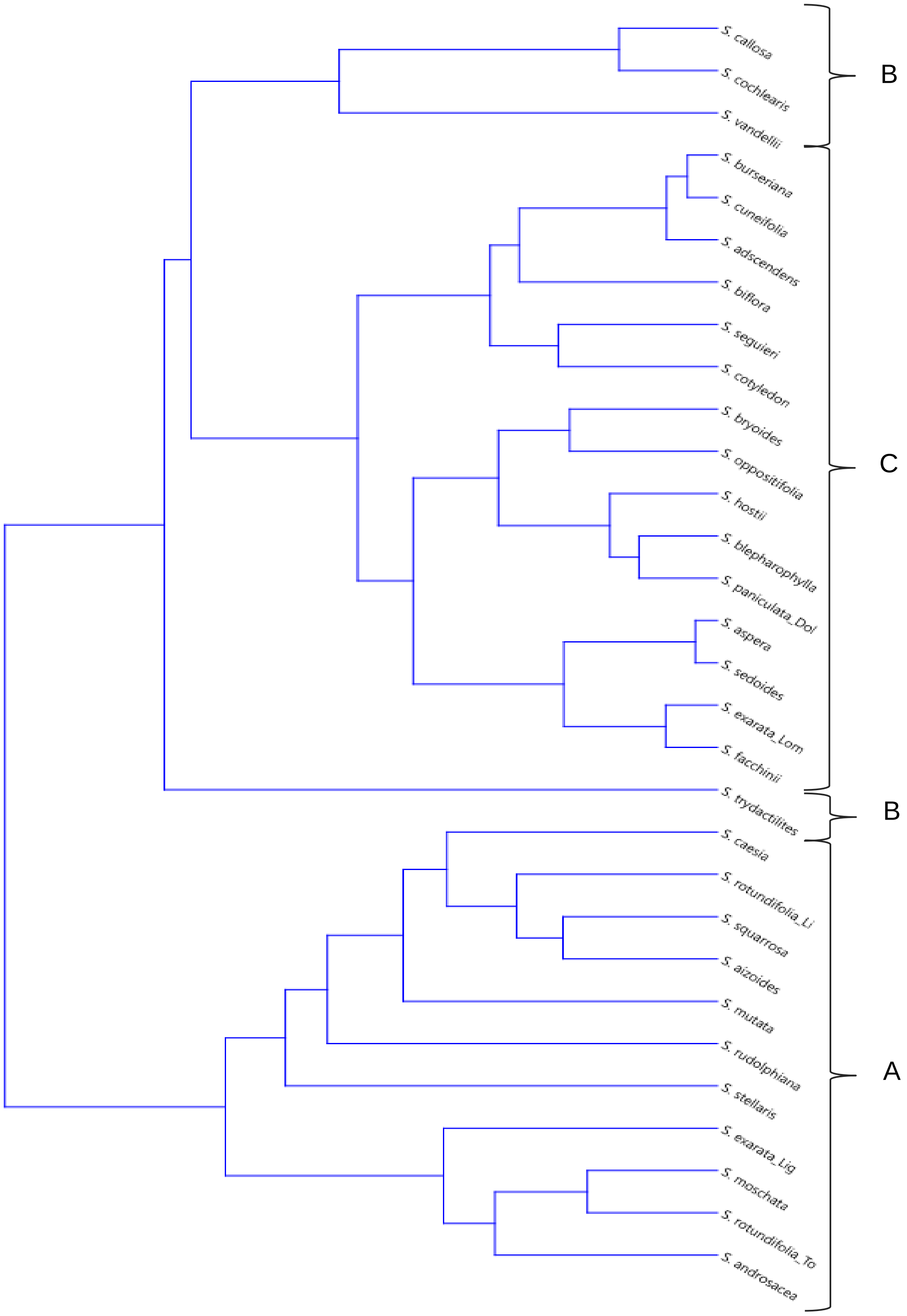


Table S3 Results for the PERMANOVA and post-hoc test for Groups defined by the cluster analysis. significant *p*-value are highlighted in bold.

PERMANOVA			
Permutation N:			10000
Total sum of squares:			1.88e^5
Within-group sum of squares:			7.17e^4
F:			21.81
p-value:			**<0.001**
Post Hoc test			
	GroupA	GroupB	GroupC
GroupA		**<0.001**	**<0.001**
GroupB	**<0.001**		**<0.001**
GroupC	**<0.001**	**<0.001**	

5. Discussion and conclusions

The first step of my research was to develop an effective way to assess the biodiversity status of alpine plant communities along a temporal gradient on GLORIA Target Regions. The methodology here proposed was developed taking into account the structure and type of data collected following the GLORIA protocol during periodic vegetation surveys. The multi-faceted analysis used, comprehensive of several diversity measures able to depict diversity from multiple point of view at once paired with Multiple site β-diversity indexes, were able to quantify diversity changes over time effectively. Overall, the diversity of the study site decreased over time, despite the increased species richness recorded in the period 2001-2015. The diversity decrement observed was mainly caused by the cover increment of graminoids and shrubs species, that reduced vegetation evenness and diversity, becoming more dominant. As the target region were free from anthropogenic impacts, I hypothesize that the plant community changes observed could be linked to climate change, as in the target area a constant increment of summer air temperature was recorded. These findings, along with the species turnover and species loss recorded at the study site, suggest the presence of an ongoing process of homogenization and biodiversity loss. If the trends observed in the study area will continue over time, a transformation of the vegetation could be expected in the next decades. Consequently, conservation activities, such as *ex/in situ* conservation actions are needed in the very next future, especially for plant species that are already declining in the studied mountain summits. The multi-faceted approach proposed in this study for the assessment of biodiversity over time could be effectively applied to the long-term vegetation data collected in the other GLORIA Target Regions. The potential application of these methods within the GLORIA network (that include more than 120 sites), could thus result in a useful tool to highlight the areas most threatened by plant diversity loss in high elevation habitats and monitoring biodiversity changes over time in alpine environment all over the world.

As observed in chapter one, several alpine plants within the GLORIA target region were characterized by clear population dynamic patterns (e.g. constantly increasing or decreasing over time). The abundance and presence/absence data of those species, collected within the Project GLORIA, were used as knowledge base for the second part of my thesis, which aimed to investigate possible relationships between alpine plant population dynamics observed in nature and their regenerative traits in the context of climate change. The results presented here highlight that species that are increasing in nature germinate at a broader spectrum of environmental conditions than those of the other groups, i.e. under snow cover and with high FGP at low temperature, especially after CS. These traits suggest that germination during early spring, right after snowmelt, may be an advantage in a warmer climate, for example, promoting seedling emergence when water is more available and drought/heat hazards are low.

Finally, the last part of my Ph.D., I investigated the relationship between seed germination and environmental cues of 28 species of the genus *Saxifraga*, with the aim to disentangle their germination response from ecological drivers and interspecific differences (i.e. differences in germination traits determined by phylogenetic distances from the species). Overall, seed germination in the genus *Saxifraga* was promoted by cold stratification and cool temperatures and it was strongly inhibited by darkness. These results indicate that *Saxifraga* species tends to germinate at the soils surface, immediately after snow melts in early spring, when water availability is high and temperatures are cool enough to prevent topsoil desiccation. However, it was possible to observe also different germination strategies that reflected the species ecological niche, i.e. requirements for water availability, soil acidity and texture. The low phylogenetic signal found for the germination responses (except light), indicate that environmental variables (both present and experienced in the past) may play a major role in determining different germination strategies, even across closely related species.

6. Additional scientific output

Beside the aforementioned paper of Chapter 2, during my PhD I was Involved in the realization of other two articles, dealing with Plant ecology, climate change and\or plant genetic resources conservation, published in international indexed journals:

I. Rogora M, Frate L, Carranza ML, Freppaz M, Stanisci A, Bertani I, Bottarin R, Brambilla A, Canullo R, Carbognani M, Chelli S, Cremonese E, Cutini M, Di Musciano M, Erschbamer B, Godone D, Iocchi M, Isabellon M, Magnani A, Matteucci G, Mazzola L, Morra di Cella U, Pauli H, Petey M, Petriccione B, **Porro F**, Psenner R, Rossetti G, Scotti A, Sommaruga R, Tappeiner U, Theurillat J-P, Tomaselli M, Viglietti D, Viterbi R, Vittoz P, Winkler M and Cerrato C (2018). Assessment of climate change effects on mountain ecosystems through a cross-site analysis in the Alps and Apennines. Science of the total environment, 624, 1429-1442.

Abstract: Mountain ecosystems are sensitive and reliable indicators of climate change. Long-term studies may be extremely useful in assessing the responses of high-elevation ecosystems to climate change and other anthropogenic drivers from a broad ecological perspective. Mountain research sites within the LTER (Long-Term Ecological Research) network are representative of various types of ecosystems and span a wide bioclimatic and elevational range. Here, I present a synthesis and a review of the main results from ecological studies in mountain ecosystems at 20 LTER sites in Italy, Switzerland and Austria covering in most cases more than two decades of observations.

We analyzed a set of key climate parameters, such as temperature and snow cover duration, in relation to vascular plant species composition, plant traits, abundance patterns, pedoclimate, nutrient dynamics in soils and water, phenology and composition of freshwater biota. The overall results highlight the rapid response of mountain ecosystems to climate change, with site-specific characteristics and rates. As temperatures increased, vegetation cover in alpine and subalpine summits increased as well. Years with limited snow cover duration caused an increase in soil temperature and microbial biomass during the growing season. Effects on freshwater ecosystems were also observed, in terms of increases in solutes, decreases in nitrates and changes in plankton phenology and benthos communities. This work highlights the importance of comparing and integrating long-term ecological data collected in different ecosystems for a more comprehensive overviewof the ecological effects of climate change. Nevertheless, there is a need for (i) adopting

co-located monitoring site networks to improve our ability to obtain sound results from cross-site analysis, (ii) carrying out further studies, in particular short-term analyses with fine spatial and temporal resolutions to improve our understanding of responses to extreme events, and (iii) increasing comparability and standardizing protocols across networks to distinguish local patterns from global patterns.

II. Müller, J. V., Berg, C., Détraz-Méroz, J., Erschbamer, B., Fort, N., Lambelet-Haueter, C., ... and Porro, F. (2017). The Alpine Seed Conservation and Research Network-a new initiative to conserve valuable plant species in the European Alps. Journal of Mountain Science, 14(4), 806-810.

Abstract: Safeguarding plants as seeds in *ex situ* collections is a cost-effective element in an integrated plant conservation approach. The European Alps are a regional centre of plant diversity. Six institutions have established a regional network covering the European Alps which will conserve at least 500 priority plant species and which will improve the conservation status of plant species in grassland communities in the subalpine, alpine and nival altitudinal belts. Targeted research will expand the knowledge of the ecology of target species. Public engagement activities will raise the awareness for the importance of specific conservation actions in the European Alps.

Additionally, I presented my research activity through three oral communications and two poster in three international, and two national, conferences:

I. Porro, F., Jiménez-Alfaro, B., Orsenigo, S., Rossi, G., Magreiter, V., Carta, A., Mondoni, A. (2019) Ecological-related germination traits in the genus *Saxifraga*. - Seed Ecology VI conference, Regensburg (DE) [Oral communication]

II. Gheza, G., Chiatante, G., Tassone, P., Odicino, M., Tescari, M.L., Nascimbene, J., Assini, S., Bogliani, G., Bracco, F., **Porro, F.,** Porro, Z. (2019) Dai boschi naturali ai boschi di impianto: confronti tra comunità licheniche ed ornitiche. - XXXII Convegno della società Lichenologica Italiana, Bologna (IT) [Poster]

III. Porro, F., Orsenigo, S., Mondoni, A. (2018) Germination responses across *Saxifraga* species suggest different patterns in western and eastern Alps.- PopBio conference, Innsbruck (AU) [Oral communication]

IV. Porro, F., Rossi, G., Mondoni A., (2018) Studio comparativo sulla germinazione e sulla longevità dei semi di piante alpine nel contesto del cambiamento climatico - XII Assemblea Nazionale e Convegno Scientifico annuale della Rete LTER-Italia, Bolzano (IT). [Oral communication]

V. Mondoni, A., Breman, E., Berg, C., Detraz-Meroz, J., Erschbamer, B., Fort, N., Lambelet-Haueter, C., Margreiter, V., Mombrial, F., Abeli, T., Pagitz, K., **Porro, F.,** Rossi, G., Schwager, P., Cesprini, M., Müller, J.V., (2017) Alpine Seed Conservation and Research Network. - XIX International Botanical Congress, Shenzen (CN). [Poster]

7. Aknowledgements

I would like to express my very deep gratitude to my tutor Andrea Mondoni, for his patient guidance, enthusiastic encouragement, useful critiques, and most of all, assistance in keeping my progress on schedule. I would also like to thank Professor Graziano Rossi, for his numerous and valuable advices and constant support.

My grateful thanks are also extended to Professor Marcello Tomaselli, Dott. Thomas Abeli and Dott. Michele Carbognani for sharing their knowelde and for their aid in data analysis; and to Dott. Maria-Tudela, and the students Alberto Quaglia and Silvano Lodetti, for their priceless help during the laboratory activities.

I wish to thank various people for their contribution to this project; Maria, Malaka, Filippo for their valuable technical support on this project; Professor Brigitta Erschbamer, Lena Nicklas, Rosa Fernàndez, Michele Di Musciano, and all the members of the "Alpine seed conservation and research Network" for their help in collecting the seed material and for the field work logistic support. A very special gratitude goes out to The David And Claudia Harding Foundation, for helping and providing the funding for this research.

Finally I am deeply indebted to my Family, Giovanna, Aldo and Federica, and my friends, Zeno, Federico, Andrea, Matteo, Nicholas, Arianna, Gabriele, G.P., Ludovico, Gianluca, Magda, Elisa and many others. You have always been there, ready to laugh, cheers and have fun, brightening my days. Thank you all, Sincerely.

Fra