Effects of climate change on alpine vegetation – functional analysis as a basis for future predictions

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Моей семье, Александру, Наталье и Марии Росбах,
которые верят в меня и поддерживают,
чем бы я не занимался и где бы я не был

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Summary

Despite broad evidence that climate change continuously effects alpine vegetation at different levels, the mechanistic understanding of these changes is still missing. The aims of the present thesis were elucidating three key aspects regarding the application of a functional approach to explore climate-induced changes of alpine vegetation. In the first part of the thesis, climate relevance of three key plant functional traits (specific leaf are (SLA), seed germination, pollen germination and pollen tube growth) was examined, in order to prove the possibility to use them as direct correlates of species distribution ranges. Second, I aimed to provide recommendations for correct application of the functional traits, considering trait intraspecific variability, trait variations at species and community levels, effect of other environmental factors on trait-climate relation and trait' phylogenetic constraints. In the last part of the thesis the functional approach as suitable tool to detect mechanisms ruling recent changes of alpine vegetation was verified.

Chapter 2 deals with the strength of the relationship between SLA and temperature at the population, species and community levels, including impacts of other environmental habitat characteristics on this relationship. The results of this chapter indicated that the intraspecific variability of SLA values was relatively low. Only 5 out of 28 studied species showed significant differences in their trait values along the temperature gradient. SLA was significantly correlated with temperature at the species ($r^2=0.04$, $p<0.001$) and community levels ($r^2=0.08$, $p<0.001$). The correlation between SLA and the mean annual temperature was modified by the effects of disturbance and soil phosphorous content, explaining 9.8% and 8.9% of the modelled variation, respectively ($r^2=0.89$, $p<0.001$). We suggest that the strong relationship between SLA and temperature provides convincing evidence that this easy-measurable trait can be used as a predictor of species distribution ranges under changing temperature conditions.

In chapter 3 I tested whether temperature requirements of pollen germination and tube growth could be used as direct correlates for predicting species distribution ranges. The results of this study revealed a strong and positive relationship between the minimal and optimal temperatures of pollen germination and temperature conditions at our collection sites ($r^2=0.41$, $p<0.01$ and $r^2=0.2$, $p<0.05$, respectively). For pollen tube growth, the minimal and optimal temperatures of the process were also significant predictors of species occurrence under the respective climatic conditions ($r^2=0.61$, $p<0.001$ and $r^2=0.57$, $p<0.001$, respectively). The strong relationship found between the temperature requirements of the progamic phase and habitat temperature could help in understanding the present species distribution along climatic gradients

from a functional perspective and assist in better projecting of future distributional range alterations caused by environmental changes.

Chapter 4 explores to which extent germination requirements of species with different geographic boundaries are related to their habitat macroclimatic characteristics. The results of the study show that initial temperature of seed germination (T_{min}) is direct correlate for predicting species distribution ranges along temperature gradient. The study reveals that T_{min} is strongly negatively correlated to habitat temperature; among the studied species T_{min} clearly increased with decreasing mean annual temperature (MAT)($r^2 = 0.57$, $p<0.001$). Considering phylogenetic biases, coevolution of seed traits as well as precipitation along with microclimatic factors did not affect the strength of this relationship. Our results suggest that the T_{min}-MAT relation can particularly provide insights into species distribution patterns, vegetation dynamics and community assembly rules along altitudinal and latitudinal gradients. We argue that including the T_{min} in species distribution models may help to improve the accuracy and specificity of predictions of vegetation shifts under global change scenarios.

The chapter 5 deals with changes in subalpine, lower and upper alpine vegetation belts in the Bavarian Alps in last 50 years. In order to reveal mechanisms standing behind the changes, functional traits, along with several common floristic and phytosociological methods, were applied. The results of

the study indicate that the direction and magnitude of the changes differed considerably among the studied communities and were dependent on initial species composition, vegetation structure and geographical context of individual vegetation belts. Although the species richness did not change significantly over last 50 years in the dense subalpine *Seslerio-Caricetum sempervirentis* communities, the immigration of species from lower altitudes and increased environmental favorability for already present thermophilous species led to increase of plant cover (+23%). The most pronounced changes were found in sparse low alpine *Caricetum firmae* communities. Due to gap availability and low competitive ability of alpine species, significant increase in species richness and plant cover (+6.4 species and +18%, respectively) were detected in this vegetation belt. The analysis revealed that in both communities species with traits that are advantageous under warmer conditions, such as higher specific leaf area, high stature and heavier seeds, significantly increased their frequencies and abundances. Floristic and vegetation changes of upper alpine communities were contrasting to those found in the study region as well as in the close mountain ridges. Despite temperature increase and available for colonization gaps in the communities, species richness (–4.7 species) and plant cover (–25%) significantly decreased. We argue that increased instability of high alpine habitats, due to upward retreat of the permafrost zone (as a consequence of recent warming), could hamper the colonization of high altitudes by montane and subalpine species, since they demand developed soils with high water-retention capacity.

Finally, in chapter 6 we conclude that functional approach has a big potential to improve understanding of climate-induced vegetation changes at high altitudes and better predict the direction and magnitude of these changes under future climate scenarios. Some aspects of future studies in this field are also discussed.

Chapter 1

General introduction

Climate change at high altitudes and the need for other research tools

It has been nearly 20 years, since Georg Grabherr, Michael Gottfried and Harald Paull from University of Wien have published a paper in *Nature* on effects of global warming on mountain plants (Grabherr, Gottfried & Pauli 1994). In the one-page paper they provided first conclusive evidences of significant effects of global warming on alpine vegetation, namely an increase of species richness during past few decades at alpine and nival summits in the Alps. After the problem of negative impacts of human-induced climate change on alpine vegetation was recognized first time at such high scientific level, alpine plant ecology has experienced tremendous growth (Figure 1). Since then, numerous studies have confirmed that ongoing climate change continuously effects alpine vegetation at different levels (Grabherr, Gottfried & Pauli 1994; Theurillat & Guisan 2001; Walther *et al.* 2002; Gottfried *et al.* 2012). Shifts in plant life-history (McKone, Kelly & Lee 1998; Mondoni *et al.* 2012), disruptions of biological relationships (Klanderud 2005; Hegland *et al.* 2009), general upward shifts of species' distributional ranges (Walther, Beißner & Burga 2005; Jurasinski & Kreyling 2007; Pauli *et al.* 2012) belong to the most often observed responses of alpine vegetation to recent global warming. The latter is particularly considered to be a serious warning, because, due to highly specific ecological conditions (Körner 1999; Nagy & Grabherr 2009) and low competitive ability of cryophilous species, it could lead to a drastic decrease of the distribution area or even extinctions of alpine species (Pauli, Gottfried & Grabherr 2003), retreat upwards and habitat loss of alpine vegetation (Thuiller *et al.* 2005; Gottfried *et al.* 2012). That is why this problem is of high importance for nature conservation and management decisions around the world, since there is now ample evidence that the recent climatic changes are affecting a broad range of mountain regions across all continents, except of Antarctic (Walther *et al.* 2002; Danby *et al.* 2011; Jump, Huang & Chou 2012; Pauli *et al.* 2012; Venn, Pickering & Green 2012).

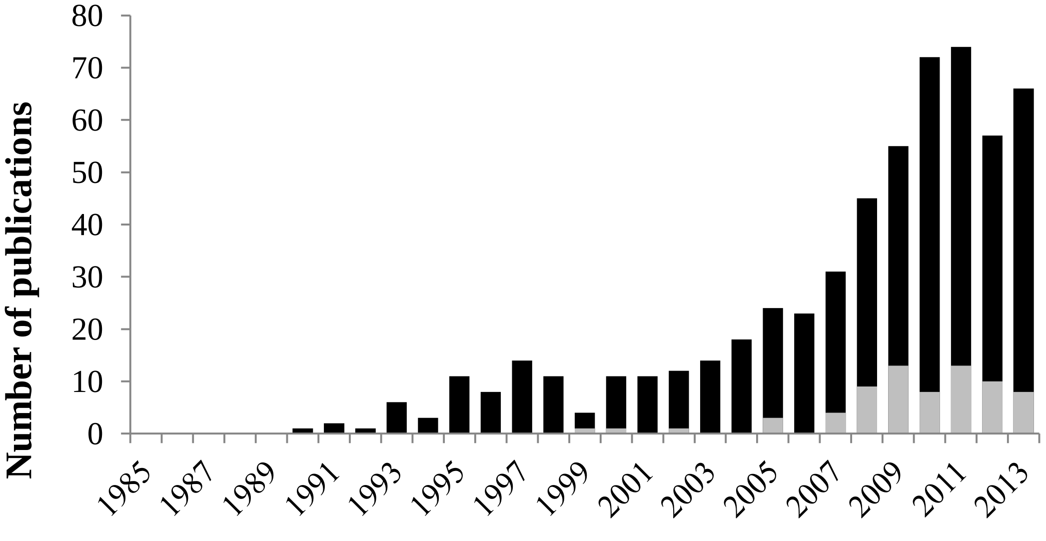


Figure 1. Number of studies on effects of climate change on alpine vegetation. Date of information gathering: September 2013. Source: ISI Web of Science. Black bars represent papers with terms climate change and alpine vegetation and the corresponding combinations in abstract of title. Grey bars represent the proportion of papers in which plant functional traits were applied (additional term to the research query "functional trait").

During the last years several research projects have been launched in order to observe, understand and predict the vegetation changes in alpine biomes (e.g. GLORIA (Grabherr, Gottfried & Pauli 2000), ITEX (Henry & Molau 1997). Among them is FORKAST, a research cooperation on impacts of climate change on different ecosystems of Bavaria (Germany), including alpine grasslands (www.bayceer.uni-bayreuth.de/forkast/). This thesis was carried out within a FORKAST-subproject (TP 12 Poschlod) with the aim to understand the mechanisms standing behind the present distribution of plant species along an altitudinal gradient, in order to provide a reliable basis for future predictions of climate-induced vegetation dynamics in the Bavarian Alps. The need for such study is explained by fact that, despite extensive evidences of high impacts of climatic change on alpine vegetation (574 papers published on this topic in last 23 years; Figure 1), there is still a gap in mechanistic understanding of these changes. Indeed, mainly biodiversity changes (e.g. number of gained and lost species, changes in species richness) and changes in vegetation (changes in plan cover, structural changes of plant communities) were employed as research methods in the majority of studies on the topic. Although both approaches allow to track the climate-induced changes in floristic and vegetation patterns over wide territories (e.g. GLORIA project (Gottfried *et al.* 2012; Pauli *et al.* 2012)), they hardly provide information on causes and mechanisms why a plant species or plant community changed its abundance and/or presence at a certain place and composition, respectively, in consequence of the environmental change.

Consequently, without a clear understanding of plant-climate relationships any conclusions on present shifts of alpine vegetation and projections of its future changes are suspect (Pearson & Dawson 2003; Thuiller *et al.* 2008).

Functional ecology meets alpine botany – a promising approach to understand vegetation changes in high altitudes

It is generally agreed that climate controls species distribution through ecophysiological processes of temperature and precipitation tolerance (Sakai 1987; Woodward & Williams 1987; Walther *et al.* 2002; Larcher 2004). To explain the controlling role of climate on a species' geographical distribution, two approaches, differing in basic concepts, are usually employed. The first approach is based on a concept that species distribution is determined by physiological capacity of adult plants to cope with critical climate events (e.g. frost, drought, etc.). For example, the poleward spread of particular vegetation type is strongly controlled by ability of plants to survive low temperatures or late frost events (Sakai 1987; Woodward 1987). In second approach the climatic control of species distribution is seen as a process which is mediated through population dynamic (Woodward 1987). In this case it is assumed that progressive reductions in probabilities of survival and production at one or number of life-cycle stages is critical in controlling distribution (Woodward & Williams 1987). For instance, Pigott and Huntley (1981) have demonstrated that the temperature sensitivity of pollen tube growth and the short period of stigmoid and stylar receptivity in *Tilia cordata* could account for the northern limit of the species in the British Isles. Although the first approach is widely used in biogeography (Sakai 1987; Woodward 1987), including modeling of potential species distribution in future (e.g. (Guisan, Theurillat & Kienast 1998; Thuiller *et al.* 2005)), it explains only a portion of large-scale distribution ranges, such as the borders of main floristic or vegetation zones, as the survival limits of plant species from different biomes are often overlapping (Sakai 1987; Körner 1999; Larcher 2004). Additionally, the geographical limits of vegetative survival may be determined by very different environmental conditions than those at other stages of the plant life cycle (Woodward 1987; Woodward 1997; García *et al.* 2000). Therefore, the second approach was selected as a theoretical framework for the thesis, because it could provide a better mechanistic base for the observed alpine vegetation responses to climate change.

As a practical framework for the thesis (Figure 2), we employed a functional approach, which connects ecophysiological responses of plant species to changing environment and both distribution patterns and vegetation dynamics through a set of common biological attributes (functional traits; FT)(Diaz & Cabido 1997; Lavorel *et al.* 1997; Westoby 1998; Weiher *et al.* 1999; Wilson, Thompson & Hodgson 1999; Lavorel & Garnier 2002; Canadell *et al.* 2007). The functional approach is based on the assumption that plants with similar biology/ecology respond to environmental changes in comparable ways, thus allowing us to reduce the enormous complexity of individual species and population needs into a relatively small number of general recurrent patterns (Diaz *et al.* 1999; Lavorel & Garnier 2002). The advantage of the functional approach is that ecosystem processes are determined by the traits and functional diversity of the dominant species and, thus, are relatively insensitive to the richness of other species (Grime 1998). In combination with the simplicity of measurements of FTs, in contrast to elaborated ecophysiological studies (e.g. photosynthetic gas exchange), it notably simplifies ecosystem service assessment and/or modelling of vegetation dynamics at any scale (Canadell *et al.* 2007).

In the last two decades a big effort has been done to approve the relevance of FTs to changing environmental factors; dozens of plant traits were found to respond to temperature, water, availability of soil resources and levels of disturbance ((Woodward & Cramer 1996; Bernhardt-Römermann *et al.* 2011; Klaiber *et al.* 2013); for detailed review see (Cornelissen *et al.* 2003)). Several broadly used functional traits such as plant life form, plant height or SLA have become very popular and have been used in the majority of environment-plant functioning related studies (e.g. (Kleyer *et al.* 2008)).

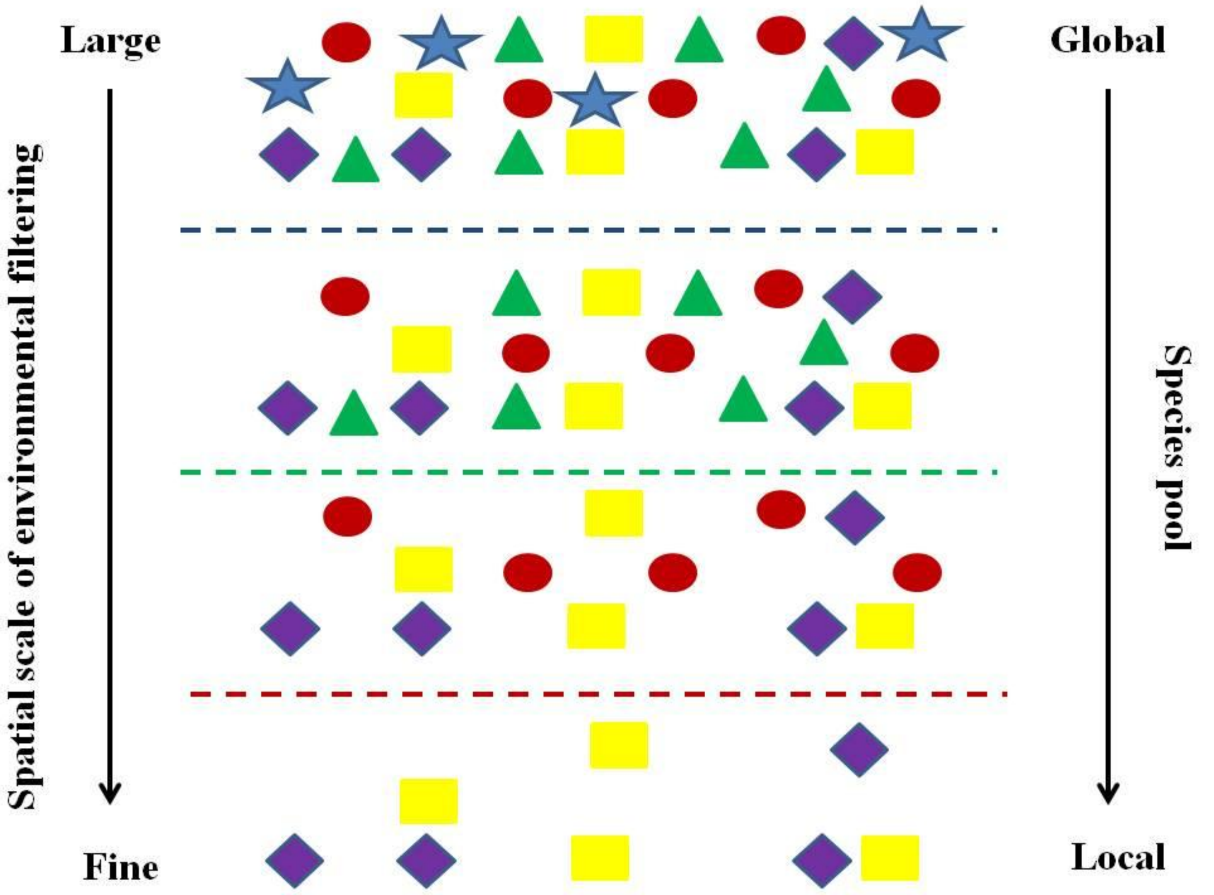


Figure 2. The graphical representation of functional approach in regard to species distribution patterns. Dashed lines are different environmental filters.

Additionally, several powerful statistical methods were developed, allowing inclusion of FTs into analysis of vegetation or flora changes by means of ordination techniques (e.g. RLQ ordination (Dolédec *et al.* 1996). Despite this popularity and widespread occurrence, the functional approach has hardly ever (however, see (Kudernatsch *et al.* 2005; Vittoz *et al.* 2008)) been applied to get a better understanding of alpine vegetation changes caused by global warming. In the existing papers pool only 13% of all studies applied functional traits to explain response of alpine vegetation to changing environment (Figure 1).

Moreover, the most of the used traits were only related to the morphology and growth rate of species. The little attention paid by alpine botanists to FTs is explained by fact that their relevance to climate is based mainly on some case studies, mainly comparing trait values of species originating from contrasting climatic conditions (Table 1). Although these comparisons aid to explain the large scale differences in climate response between species with different distributional ranges, such as lowland and alpine species, they are not helpful at regional and local scales (e.g. species with distribution within one vegetation belt). Meta-analyses of trait variation along climatic gradients (e.g. SLA, (Poorter *et al.* 2009)), with more species included, therefore, did not improve the strength of the trait-climate relationships, because they usually disregarded all important sources both internal and external of the trait variation (Ackerley *et al.* 2002; Vile *et al.* 2006; Albert *et al.* 2010; Lepš *et al.* 2011). Another reason which explains low interest of alpine botanists to functional traits is that mainly vegetative traits (whole plant, leave and stem traits) are applied to determine plant-climate interactions and regenerative traits are often remain underrepresented (Kleyer *et al.* 2008; Kattge *et al.* 2011; Pérez-Harguindeguy *et al.* 2013). However, recent studies demonstrate that reproductive stages of plant life cycle such as fecundation, seed germination and seedling recruitment may be more temperature sensitive and are essential to understand plant distribution over the landscape (Grubb 1977; Diaz & Cabido 1997; Fenner & Thompson 2005; Bykova *et al.* 2012; Poschlod *et al.* 2013).

Table 1. Climate response of functional traits. See also (Kiebacher 2008; Cornelissen *et al.* 2003) for detailed reviews.

Life cycle stage	**Related fnctional trait**	**Climate response**		**Reference**
		Large scale	**Local scale**	
Seed bank	Seed shape	?	?	
	Seed weight	+	?	(De Frenne *et al.* 2013)
	Seed coat	?	?	
	Seed longevity	+	+	(Probert, Daws & Hay 2009)
Establishment	Seed dormancy	+	+	(Grime *et al.* 1981; Baskin & Baskin 1998)
	Seed germination requirements	+	?	(Thompson 1968; Trudgill, Squire & Thompson 2000)
Vegetative	Plant life form	+	+	(Mahdavi, Akhani & Maarel 2013)
	Canopy height	+	?	(Venn *et al.* 2011)
	Leaf size	+	+	(Diaz & Cabido 1997)
	Leaf mass	+	?	(Koerner *et al.* 1989)
	Specific leaf area	+	?	(Venn *et al.* 2011)
	Leaf dry matter content	+	?	(Wright & Westoby 2002)
	Woodness	?	?	
Reproduction	Flowering time	?	+	(Cornelius *et al.* 2013)
	Pollination mode	+	?	(Diaz & Cabido 1997)
	Fecundation requirements	?	?	
	Seed crop size and frequency	+	?	(Diaz & Cabido 1997)

Table 1. Climate response of functional traits (continued).

Life cycle stage	**Related fnctional trait**	**Climate response**		**Reference**
		Large scale	**Local scale**	
Dispersal	Fruit type	?	+	(Magalì *et al.* 2013)
	Diaspore morphology	?	+	(Magalì *et al.* 2013)
	Dispersal potential	+	?	(Tackenberg & Stöcklin 2008)
Survival	Frost-tolerance	+	+	(Neuner *et al.* 2012)
	Clonality	?	+	(Wellstein & Kuss 2011)
	Bud banks	?	?	
	Maximum age	+	+	(Schweingruber & Poschlod 2005)

Therefore, more research on regeneration processes is urgently needed to fully reveal processes controlling species distribution and abundance (Walck *et al*. 2011; Bykova *et al*. 2012; Poschlod *et al*. 2013).

Thesis outline

The present thesis aims at elucidating three key aspects regarding the application of a functional approach to explore climate-induced changes of alpine vegetation. First, I examined climate relevance of several key FTs (SLA, seed germination, pollen germination and pollen tube growth), in order to prove the possibility to use them as direct correlates of species distribution ranges. The particular emphasis was placed on temperature relevance of the selected traits, since temperature is the most important driver of species distribution in cold habitats (Woodward & Jones 1984). Second, I aimed to provide recommendations for correct application of the FTs, considering trait intraspecific variability, trait variations at species and community levels, effect of other environmental factors on trait-climate relation and trait' phylogenetic constraints. Finally, I verified the functional approach as suitable tool to detect mechanisms ruling recent changes of alpine vegetation.

In the following an overview is provided about the topics that are addressed in the individual chapters and how they are linked to the aims of our study:

Chapter 2 explores the strength of the relationship between SLA and temperature at the population, species and community levels, including impacts of other environmental habitat characteristics on this relationship. Based on an extensive data set of original SLA values, I tested whether this trait can be used in further studies as an indicator of plant performance under changing temperature conditions. The knowledge on strength of the SLA-temperature relationship at different levels is essential for practical applications of the traits, since it considers effects of internal and external sources of the trait variation.

In **chapter 3** I aimed to test whether temperature requirements of pollen germination and tube growth could be used as direct correlates for predicting species distribution ranges. Knowledge regarding the ecophysiological mechanisms that control the geographical ranges of plant species is scarce.

Therefore, this study could aid to reach a better understanding of the existing patterns of plant species distribution and to improve the accuracy and specificity of predictions of vegetation shifts under global change scenarios.

After fecundation and seed production, seed germination and seedling establishment are another two highly vulnerable stages of plant life cycle. Therefore, in **Chapter 4** I explored to which extent germination requirements of species with different distributional ranges are related to their habitat macroclimatic characteristics. Character and strength of the relationship between initial temperature of seed germination and habitat temperature particularly investigated in this study could reveal the role of germination ecology in mechanistic understanding of species distribution patterns, vegetation dynamics and community assembly rules along environmental gradients.

In Chapter 5 I verified the functional approach as an additional tool to common floristic and phytosociological methods, to reveal mechanisms standing behind climate-induced vegetation changes in the Bavarian Alps in last 50 years. The application of traits could help to explain the often reported patterns of floristic and vegetation changes from functional point of view.

Finally, the results of the previous chapters were reviewed with regard to their implications for application of functional approach in alpine botany (**Chapter 6**: Conclusion and perspectives).

Chapter 2

Specific leaf area correlates with temperature: new evidence from trait variation at the population, species and community levels

Abstract

Previous studies indicate that temperature could explain only a part of the variation of specific leaf area (SLA). We assume that this is because 1) intraspecific variation of SLA values were not considered, 2) studies were focused on species level and 3) this relation was tested on single environmental gradients regardless of associated factors potentially determining complex responses of the trait.

Using originally measured SLA values for 202 species occurring in 29 calcareous grasslands along the temperature gradient, we explored the intraspecific (population), interspecific (species) and community-weighted variations in SLA and investigated the relative impact of soil moisture content, soil depth, soil fertility, disturbance and potential solar radiation on SLA variation along the temperature gradient.

The intraspecific variability of SLA values was relatively low. Only 5 out of 28 studied species (18%) showed significant differences in their trait values along the temperature gradient. SLA was significantly correlated with temperature at the species ($r^2=0.04$, $p<0.001$) and community levels ($r^2=0.08$, $p<0.001$). The correlation between SLA and the mean annual temperature was modified by the effects of disturbance and soil phosphorous content, explaining 9.8% and 8.9% of the modelled variation, respectively ($r^2=0.89$, $p<0.001$).

The detected variation in SLA along the temperature gradient provides strong evidence that this trait can be used in further studies as an indicator of plant performance under changing temperature conditions. We argue that a strong SLA-temperature relationship is achieved when community-weighted trait values are used and when intraspecific variability as well as the effects of environmental parameters other than temperature are considered.

Introduction

Specific leaf area (SLA, one-sided leaf area per unit of dry mass) is one of the most frequently used functional traits in ecological studies (Westoby 1998; Weiher *et al*. 1999; Grime 2002). Due to its positive and linear scaling with the relative growth rate (RGR) of plants (Poorter & Remkes 1990; Cornelissen *et al*. 1996; Hunt & Cornelissen 1997; Reich *et al*. 1999; Villar *et al*. 2005), SLA has been considered in both theoretical and applied studies as a key trait explaining the distribution of species along environmental gradients (Grime 1974; Lavorel *et al*. 1997; Wilson *et al*. 1999; Garnier *et al*. 2004; Poorter *et al*. 2009). SLA values have been found to be dependent on differences in irradiation levels (Meziane & Shipley 2001), soil fertility (Poorter & De Jong 1999; Garnier *et al*. 2004), water supplies (Reich *et al*. 1999), carbon dioxide (Larigauderie *et al*. 1988) and ozone concentrations (Bussotti 2008), disturbance (Kleyer 1999) and land-use types (Diaz *et al*. 1999). In addition to explaining the locations of species along a resource gradient or changes in the species composition due to varying levels of resource availability, SLA reflects a number of important ecosystem properties such as productivity (Poorter & De Jong 1999; Garnier *et al*. 2004), litter decomposition (Cornelissen *et al*. 1999) and flammability (Lavorel & Garnier 2002). The global relevance of this functional trait has been confirmed to be independent of taxonomic affinity through analyses encompassing different floras from different biomes and biogeographic regions (Reich *et al*. 1999; Diaz *et al*. 2004). Thus, SLA is an easily measured, universally applicable tool for estimating the responses of single plant species, communities or whole ecosystems to environmental changes (Diaz & Cabido 1997; Hodgson *et al*. 1999; Cornelissen *et al*. 2003; Diaz *et al*. 2004; Vile *et al*. 2006).

It is generally accepted that SLA is correlated with the temperature conditions of a habitat (Woodward 1983; Körner 1999; Cornelissen *et al*. 2003; Luo *et al*. 2004; Wright *et al*. 2004; Poorter *et al*. 2009; De Frenne *et al*. 2013). Reduced ambient temperatures and increased temperature stress along altitudinal or latitudinal gradients lead to species turnover in plant communities, from fast-growing species with high temperature thresholds for cell extension to slow-growing species that exhibit cell extension at lower temperature thresholds (Woodward *et al*. 1986; Körner & Woodward 1987). Low rates of cell expansion relative to the rates of cell wall and protein synthesis in elongating cells as a consequence of slow growth rates (Atkin *et al*. 1996; Körner 1999) lead to a large number of small cells per unit area and, accordingly, smaller and denser leaves on plants in cold habitats (Atkin *et al*. 1996; Poorter *et al*. 2009). Additionally, the accumulation of large pools of non-structural carbon (e.g., soluble and non-soluble sugars) and high investment in (hemi)cellulose-rich sclerenchymatic tissues, which assist in coping with the frost and wind experienced at high altitudes and latitudes, can also contribute to a higher leaf density on alpine and arctic plants (Woodward 1983; Atkin *et al*. 1996; Körner 1999). Therefore, it is assumed that species with low SLA values will mainly be found in the cold part of temperature gradients, whereas species with relatively high SLA values should be largely restricted to the sites exposed to higher temperatures (Woodward 1983; Grime 2002; Poorter *et al*. 2009).

Although SLA has been proposed to reflect plant responses to climatic changes (Cornelissen *et al*. 2003; De Frenne *et al*. 2013), a close examination reveals that the link between SLA and temperature has never been systematically evaluated. The existing studies display several limitations that may affect the strength and, thus, the predictive power of the SLA-temperature relationship:

Due to plastic responses or adaptive differentiation, similar to many other leaf traits, SLA may exhibit substantial levels of intra-specific variation along environmental gradients (Albert *et al*. 2010; Auger & Shipley 2013).

Despite the fact that species differ with respect to their responses to changing environmental conditions, the trait values of single species have mainly been used to estimate the relationship between temperature and SLA (Woodward 1979; Atkin *et al*. 1996; Luo *et al*. 2004; Milla *et al*. 2009; for a detailed review, see Kiebacher 2008). However, ignoring important aspects of the variation and distribution of a trait within a site may significantly modify the trait-environment relationship (Ackerly *et al*. 2002; Ackerly & Cornwell 2007).

Because the SLA-temperature relationship is usually tested along complex environmental gradients, the direct effect of temperature on SLA is often confounded by the influence of other environmental factors, such as soil fertility (Canadell *et al*. 2007; Kőrner 2007; De Frenne *et al*. 2013).

Therefore, it is not surprising that in previous studies, including global syntheses (Wright *et al*. 2004; Ordoñez *et al*. 2009; Poorter *et al*. 2009), the correlation between SLA and temperature has been found to be weak.

The aims of the current study were to test the strength of the SLA-temperature relationship at three different levels (population, species and community) along a temperature gradient and to estimate the relative impact of habitat characteristics (besides temperature) on the variation of SLA. More specifically, we measured SLA values in a total of 202 species found in 29 calcareous grasslands located along an altitudinal gradient (1400 m difference in altitude, with a mean annual temperature range of 4°C) in the Bavarian Alps (Germany). We analysed the data with respect to (i) the intraspecific variation of SLA associated with changes in the mean annual temperature (MAT) (population level), (ii) the interspecific variation of SLA associated with MAT (species level) and (iii) the variation in community-weighted SLA values associated with MAT (community level). In a final model (iv), we estimated the effect of habitat characteristics such as soil nutrients, disturbance levels and irradiation in addition to temperature on community-weighted SLA variations.

Materials and methods

Altitudinal gradient and characteristics of the study sites

We selected an altitudinal gradient as a suitable tool for estimating trait variations because there is clear evidence that the mean annual temperature is strongly negatively altitudinal elevation (0.6K per 100 m or shortening of the vegetation period by 3-5 days per 100 m; see also Appendix 1) (Sakai 1987; Diaz & Cabido 1997; Körner 1999; Körner 2007).

Field work was carried out in Berchtesgaden National Park (Bavaria, Germany), which is situated in the Bavarian Alps (northern part of the Calcareous Alps) and is approximately 200 km² in size. The relief of the National Park can be characterised as typically alpine, with steep mountain peaks composed of

Triassic lime and dolomite rocks (Marke *et al*. 2013). The climate in the region is a typical mountain climate, showing a large decrease in mean annual air temperatures from +7° to –2°C (from 603 to 2713 m above sea level (a. s. l.), respectively). The mean annual precipitation varies from year to year, ranging from approximately 1500 to 2600 mm per year (Marke *et al*. 2013).

All measurements were performed at 29 sites located along a gradient from 641 to 2032 meters a. s. l. Every site, with a size of approximately 1 ha, was characterised in terms of its vegetation composition, climate (mean annual temperature (MAT), mean annual precipitation), soil moisture content, soil depth, soil fertility (potassium, phosphorous and total nitrogen content) and potential solar radiation. The last parameter was selected as an aggregate measure of site's irradiation conditions because it shows a good correlation with UV-B radiation (Németh *et al*. 1996) and photosynthetically active radiation (Meek *et al*. 1984; Papaioannou *et al*. 1993). The potential solar radiation was calculated from the slope and aspect of the study sites using a solar radiation calculator (http://www.meteoexploration.com). Only estimates obtained for the summer solstice were used. Additionally, estimates of the level of disturbance (at two levels: disturbed by, e.g., heavy grazing and avalanches, versus undisturbed) were recorded at every study site.

The vegetation composition of the sites was characterised through surveys conducted from May to August in 2009. To carry out these vegetation surveys, we set up ten 1 m x 1 m plots at each site. In every plot, we recorded the presence of each vascular plant species and estimated their percentage of cover. The relative abundance of a species at a site was then calculated as the mean value of its abundance in all plots.

Data on the mean annual temperature and precipitation were obtained from the weather stations located in the National Park and are presented as the mean values for the last 7 years of observations. The soil moisture content was measured once at all sites in the middle of the vegetation period (August 2009) within 3-5 days after the last rainfall event using an HH2 moisture meter (Delta T Devices, Cambridge, UK). Soil depth was estimated by thrusting an iron rod with a 0.6 mm diameter into the soil. Both the soil moisture content and soil depth are presented as the mean values of 25 random measurements per site.

All soil samples were collected within two weeks at the end of the vegetation season (September 2009). At each site, six random pits were dug, and the soil from a depth of 5-10 cm was collected, thoroughly mixed, air dried, sieved (2 mm) and subsequently analysed. Plant-available phosphorus and potassium were extracted from the collected soil samples with a calcium acetate lactate solution (VDLUFA 1991). Phosphorus contents were determined using a UV-visible spectrophotometer (*Thermo-Spectronic UV-1* model, Thermo Electron Corporation, USA) after visualising the phosphorus content with ammoniumheptamolybdate. Potassium contents were determined using an atomic absorption spectrometer (Solaar AA model, Thermo Elemental, USA). Total nitrogen was quantified via gas chromatographic measurement with an element analyser (Vario EL III model, Elementar Analysensysteme GmbH, Germany). The data on phosphorus, potassium and nitrogen contents are presented as the mean values of the six replicates per site.

Specific leaf area measurements

Based on the vegetation surveys, we first selected all species showing a relative abundance of greater than 1% of the total vegetation cover of a plot (in total 202). The species' relative abundances among plots were compared, and the plot with the highest value (i.e., showing optimal ecological conditions) was taken as the site where SLA measurements of single species were subsequently carried out.

To estimate the intraspecific variation of the examined trait with temperature, SLA values were obtained for the 28 most abundant species from six to twelve additional locations. In all cases, the collection sites were spanned more than 1000 meters along the altitudinal gradient.

Specific leaf area was measured following standardised protocols (Garnier *et al*. 2001; Cornelissen *et al*. 2003; Kleyer *et al*. 2008). For these measurements, three fully expanded, illuminated, non-damaged leaves with petioles were collected from 10 randomly selected full flowering individuals per site. In some cases (for rare or threatened species), only two or three leaves from five individuals were collected. Due to the strong effect of the water leaf status on SLA values, the collected plant material was stored in sealed humidified plastic bags in a refrigerator at a temperature of +5°C prior to processing (Garnier *et al*. 2001). The storage time did not exceed 48 hours (Cornelissen *et al*. 2003). Following rehydration, the leaves were scanned on a flat-bed scanner (HP Scanjet G4010) at a resolution of 600 dpi, and the leaf area was measured with the aid of ImageJ software (Abramoff et al. 2004). Then, the leaves were oven-dried at 60°C for 72 hours and weighed. SLA is given by the ratio of the fresh leaf area to the leaf dry mass expressed in mm^2 mg^{-1}.

Data analysis

Relationship between SLA and temperature at the population level

The within-species SLA variation associated with MAT at the collection sites was analysed using separate linear models for each species. Nonlinear terms ($SLA \sim MAT + MAT^2$) were also considered in these models because a trait-environment relationship can be non-linear when the effects of the species composition on community dynamics and ecosystem functioning are not additive (e.g., plant-soil feedback, complex biotic interactions, functional complementarity; Diaz *et al*. 2007). In cases where the quadratic term was not significant, the model was simplified. The model assumptions (a normal distribution of residual errors and homogeneous variances) were met in all cases.

Relationship between SLA and temperature at the species and community levels

Prior to estimating the variation of SLA associated with temperature at the species level, we calculated an estimated value of *K*, which is a parameter indicating phylogenetic correlation within a trait that ranges from zero to one (Blomberg *et al*. 2003). Values that are not significantly different from 0 suggest that there is no phylogenetic signal in a trait, while a value of one indicates that species traits are distributed as expected under a Brownian model of evolution. The calculation of the *K* values was based on a dated phylogeny of a large European flora (Durka & Michalski 2012) and was implemented in the R statistical environment (*picante* package) (R Development Core Team 2012; Kembel *et al*. 2010).

To test the correlation between SLA and temperature at the species level, single SLA values for all selected species (see above) were associated with the corresponding MAT at the collection sites.

Community-weighted SLA values were computed as the mean trait values weighted by the relative abundance of each species. The associations between community-weighted SLA and MAT were tested using linear models following the procedure described above.

Effect of environmental factors on SLA

Because the aggregated trait values of species that co-occur in a local community better reflect the integrated effects of multiple environmental factors

(Ackerly & Cornwell 2007), only community-weighted SLA values were used to estimate the effect of habitat characteristics on the SLA-temperature relationship. To test the relative effects of environmental factors on community-weighted SLA values, we set up a full linear model. As explanatory variables for SLA, we included MAT, annual precipitation, soil moisture content, soil depth, soil potassium, phosphorus and total nitrogen contents, potential solar radiation and disturbance levels as both simple and quadratic terms. Colinearity was not a problem, as the explanatory variables were only weakly correlated with each other (Appendix 2). To obtain estimates of the relative effects of all environmental factors, these factors were scaled to zero mean unit variance prior to analyses. The full model was reduced via backward selection of the least significant variables until we achieved the minimal adequate model (Crawley 2007). Following model fitting, the model requirements (a normal distribution and homogeneous variances in the residuals) were checked.

All statistical calculations were performed with the open source software R, version 2.14.0 (R Development Core Team 2012).

Result

The characteristics of the collection sites are presented in Appendix 1. The specific leaf area values of the studied species ranged from 6.32 (*Erica herbacea*, evergreen dwarf shrub) to 48.61 mm^2 mg^{-1} (*Myosotis sylvatica*, perennial forb), displaying a median of 22.01 mm^2 mg^{-1}. The 5th and 95th percentiles of the overall distribution of our data showed that the SLA values of the most common species in the study area ranged from 12.16 to 36.4 mm^2 mg^{-1}.

The community-weighted values of the investigated plots (Appendix 3) ranged from 11.15 (site 25, 2032 m a.s.l.) to 26.15 mm^2 mg^{-1} (site 26, 960 m a.s.l.).

Variation of SLA with temperature among populations

The SLA values of five (*Gentiana verna*, *Potentilla erecta*, *Ranunculus montanus*, *Thymus pulegiodes* and *Tofieldia calyculata*) of the 28 investigated species were significantly correlated with MAT (see Table 2 for statistical test values). Four of these species showed significant positive correlations with MAT, whereas the SLA of *Ranunculus montanus* was negatively correlated with MAT. Trends were also detected in the relationship between SLA and MAT ($p<0.1$) for *Galium anisophyllon* and *Phyteuma orbiculare*.

Relationship between SLA and temperature at the species and community levels

The low and non-significant *K*-value ($K=0.15$, $p=0.31$) calculated for the dataset including all single SLA values indicated that the variation of this trait is not phylogenetically constrained.

The results of the linear models associating species and community-weighted SLA values with MAT are presented in Figure 3 and 4. At the species level, we revealed a significant negative (though very weak) correlation between SLA and the mean annual temperature ($r^2=0.04$, $F=71.32$, $df=1522$, $p<0.001$).

Additionally, a great deal of variation was observed among the SLA values obtained at each site (Figure 3). We also detected non-linear associations between MAT and community-weighted SLA values ($r^2=0.8$, $F=52.58$, $df=27$, $p<0.001$).

Effect of environmental factors on community-weighted SLA values

The minimal adequate model revealed that in addition to the mean annual temperature (with both linear and quadratic components included), the disturbance level and soil phosphorous content also had a significant effect on the community-weighted SLA values ($r^2=0.89$, $F=47.63$, $df=27$, $p<0.001$, see model results in Table 3). The mean annual temperature explained 70.3% of the variation in SLA, while the disturbance level and soil phosphorous content explained 9.8 and 8.9%, respectively. Figure 5 graphically displays the fit of the model results.

Table 2. Intraspecific variation in the SLA values of the 28 investigated species along the temperature gradient. The altitudinal distribution, SLA range, mean and standard deviation values and the number of sites are given for each species. The Pearson correlation coefficient, r, and its p values were obtained from linear models testing the relationship with temperature. Significant results are shown in bold.

Species	**Altitudinal distribution (meters, a.s.l.)**	**SLA (mm^2 mg^{-1})**			**Number of collection sites**	**r**	**p**
		Range (min – max)	**Mean value**	**Standard deviation**			
Anthyllis vulneraria ssp. alpestris	600-2300	17.4 – 33.4	24.5	6.1	8	-0.32	0.44
Aster bellidiastrum	600-2000	13.6 – 34.4	22.8	6.6	7	-0.5	0.26
Briza media	100-1800	20.4 – 34	27	4.6	7	0.53	0.23
Campanula scheuchzeri	900-2400	18.3 – 33	22.5	4.6	12	0.16	0.61
Carex firma	800-2600	7.5 - 17	11.8	3.35	10	-0.03	0.93
Carex sempervirens	1300-2400	12 – 28.5	18	5.4	8	0.46	0.26
Dactylis glomerata	100-1800	22.7 – 33.4	27	3.4	13	0,3	0.32
Dryas octopetala	1600-2700	8.4 – 17.3	12.9	4	7	0.1	0.83
Erica herbacea	100-1700	6.3 –18.1	10.4	4.7	6	-0.32	0.53

Table 2 continued

Species	Altitudinal distribution (meters, a.s.l.)	SLA (mm^2 mg^{-1})			Number of collection sites	r	p
		Range (min – max)	Mean value	Standard deviation			
Galium anisophyllon	500-2500	20.8 – 25.	23	1.5	7	0,72	0.07
Gentiana clusii	400-2400	9.1 – 14.2	11.2	2.1	7	-0.12	0.79
Gentiana verna	400-2600	12.3 – 17.3	15.9	1.7	9	0.68	**0.04**
Globularia cordifolia	600-1800	6.9 – 18.5	12.5	4.3	6	0,39	0.45
Helianthemum nummularium ssp. grandiflorum	100-2400	13 – 19	15.9	2.6	7	-0.14	0.77
Hypericum maculatum	600-1800	22.6 – 37.2	29.8	5.4	7	0.48	0.28
Leontodon hispidus s.l.	100-2000	25.2 – 32.5	29.1	2.2	7	-0.49	0.27
Lotus alpinus	600-2000	16 – 28	22.9	3.7	12	-0.25	0.44
Phyteuma orbiculare	600-2200	18.6 – 25.8	21.7	2.6	6	-0.77	0.08
Pinguicula alpina	600-2400	27.6 – 37.4	32.6	3.9	6	-0.63	0.18
Potentilla erecta	100-2200	22.4 – 35	26.7	4.1	11	0.6	**0.05**
Ranunculus montanus	1500-2500	20.5 – 31	23.4	3.3	11	-0.86	**< 0.001**

Table 2 continued

Species	Altitudinal distribution (meters, a.s.l.)	SLA (mm^2 mg^{-1})			Number of collection sites	r	p
		Range (min – max)	Mean value	Standard deviation			
Sesleria albicans	400-2600	16.4 – 23.1	19.6	2.3	11	-0.26	0.44
Soldanella alpina	1000-2900	9.7 – 12.6	11.2	1.2	8	0.46	0.25
Thymus pulegiodes	100-1800	18.2 – 28.2	23.1	3.8	7	0.78	**0.04**
Tofieldia calyculata	400-2100	13 – 20	15.9	2.9	6	0.82	**0.05**
Trifolium pratense	100-1800	19.4 – 28.1	23.9	2.9	8	0.08	0.86

Discussion

The obtained results show that specific leaf area is significantly related to temperature. However, the strength of this correlation varies considerably depending on the scale of investigation. Analysis of the variation in SLA with changing temperatures at the population level indicated that 18% (5 out of 28) of the investigated species tend to show differences in SLA values along the examined temperature gradient (Table 2). Significant changes in SLA values associated with temperature were detected for all species (except for *Ranunuculus montanus*). Although our sampling strategy did not allow us to distinguish between different phenotypes and genotypes, we suspect that the source of the intraspecific variability observed in our study is genotypic differentiation. All species for which intraspecific variability was detected show a high level of genetic variability: they either belong to taxonomically complex species with indistinct morphological differences among microspecies (e.g., *Thymus pulegioides;* Morales 2002), or they tend to present different ecotypes in contrasting ecological conditions (e.g., *Potentilla erecta*; Richards 1973; Watson & Fyfe 1975; Leht & Paal 1998).

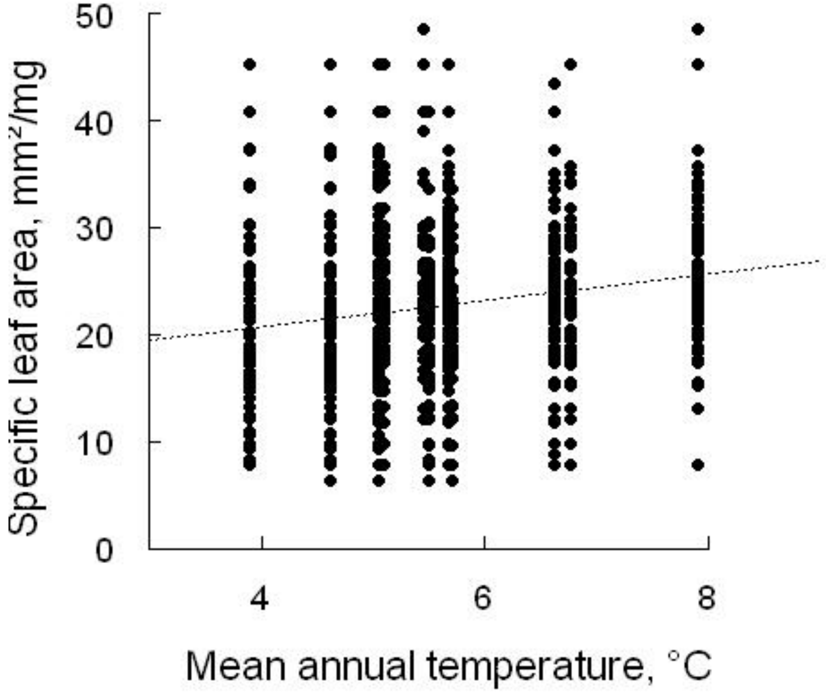


Figure 3. Correlation between SLA values and the mean annual temperature at the species level

($r^2=0.04$, F=71.32, df=1522, $p<0.001$).

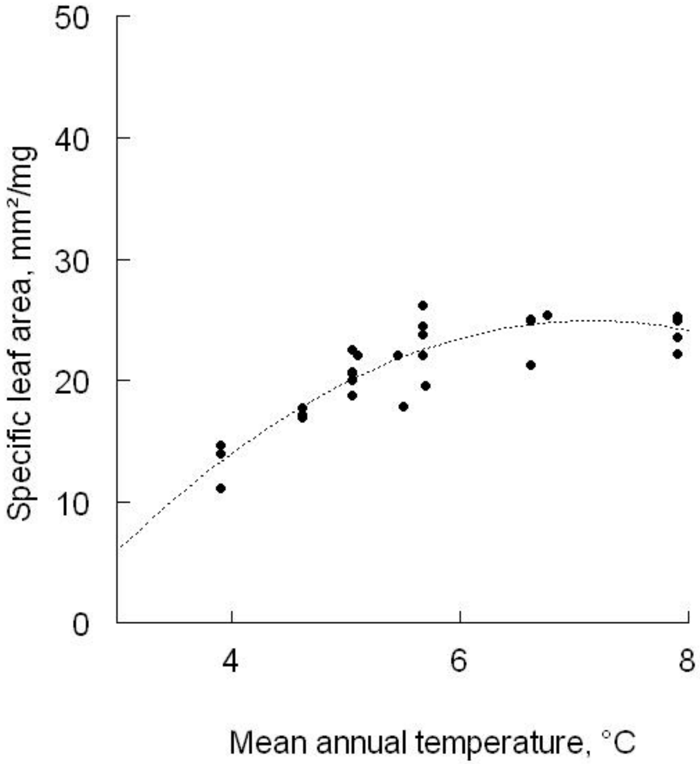


Figure 4. Correlation between SLA values and the mean annual temperature at the community level (including SLA values weighted with the abundances of the species in the community)

($r^2 = 0.8$, F=52.58, df=27, $p<0.001$).

Regardless of the source of the trait variation at the population level, these findings highlight the fact that intraspecific variability can considerably contribute to the SLA-temperature relationship. Therefore, we agree with the findings of previous studies (Albert *et al*. 2010; Lepš *et al*. 2011; Auger & Shipley 2013) indicating that incorporating intraspecific variability will improve the detection of the trait composition response to temperature variability.

The considerable decline of temperatures along an altitudinal gradient leads to species turnover and, as a result, changes in the community structure. Lowland species with high SLA values are the first species to show decreased dominance in communities along such gradients and are progressively replaced by upland species with a lower SLA. Good examples of this phenomenon in our dataset include the rapidly growing species *Arrhenatherum elatius* (SLA=29.37 mm^2 mg^{-1}) and *Leontodon hispidus* (SLA=30.21 mm^2 mg^{-1}), which dominate under lowland conditions and are absent at high-altitude sites. At these sites, these species are replaced by slow-growing alpine species such as *Carex firma* (8.24 mm^2 mg^{-1}) and *Dryas octopetala* (12.30 mm^2 mg^{-1}). Although this replacement was reflected at both the species and community levels, the variation of single-species SLA values along the temperature gradient was much lower, but still statistically significant, compared to the community-weighted values (r^2=0.04 vs. r^2=0.8, respectively).

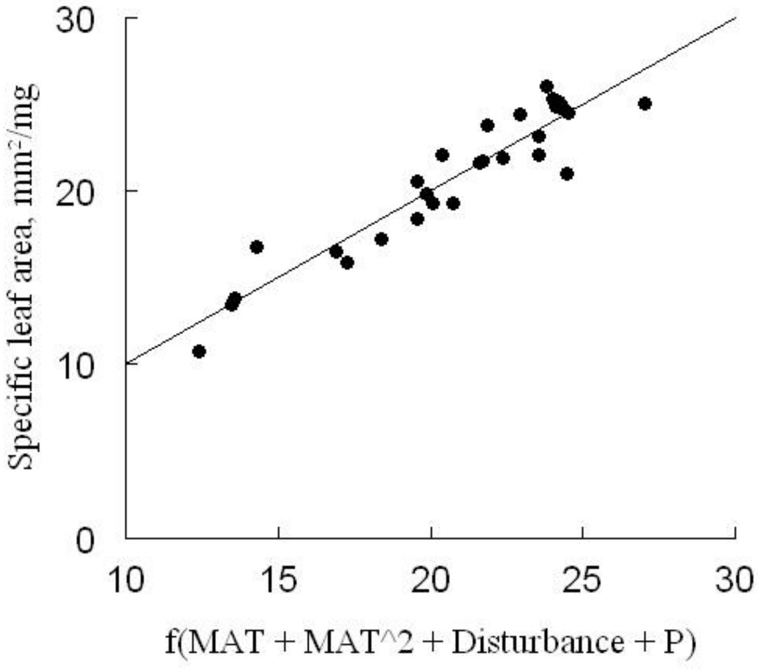


Figure 5. Graphical representation of the results of the linear model investigating the effect of environmental factors on community-weighted SLA values ($r^2 = 0.89$, F=47.63, df=27, $p<0.001$). Table 3 provides the model estimates. MAT and MAT^2 are the mean annual temperature and its quadratic component, respectively.

Moreover, the trait variation observed at the species level indicates that a variety of plants with considerably different leaf morphologies are successful under each MAT (Figure 3). This difference can be explained by the fact that at the species level, the trait values of single species were considered to exhibit equal relevance. However, due to the gradual species replacement along the temperature gradient, species occurring under contrasting temperature conditions may coexist together in a single plant community, especially in the middle portion of the gradient. Additionally, heterogeneous habitats, or more specifically, micro-habitats with distinct abiotic conditions, enable species to occur outside of their ecological optimum. In these micro-habitats, lowland species may still occur in the alpine belt on southern slopes, and alpine species may find suitable growing conditions at low altitudes, such as on rocky outcrops (see outliers at 4 and 6°C MAT in the Figure 3). Thus, the single trait values do not consider species' abundances, though they represent excellent indicators of the environmental favourability for a species (Grime 1998). In this context, the community-weighted values of the trait reflect the ability of a species to pass through a filter (as do single values) as well as referring to the role of a species in the community (Grime 1998; Garnier *et al*. 2004; Canadell *et al*. 2007; Suding *et al*. 2008; Klumpp & Soussana 2009).

Although altitudinal gradients are a perfect natural laboratory for investigating plant trait-temperature responses (Körner 2007), they encompass variations not only in atmospheric temperature but also in soil fertility, water supplies, irradiance and the level of disturbance (Canadell 2007; Kőrner 2007). Because specific leaf area reflects resource availability, these factors may also affect the variability of this trait (Ackerly *et al*. 2002; Garnier *et al*. 2004; Poorter *et al*. 2009). Our results showed that in addition to the strong positive effect of temperature that was detected, disturbance and soil phosphorous contents were also significantly associated with changing SLA values along the altitudinal gradient.

Table 3. Effect of environmental factors on community-weighted SLA values, as deduced from the linear model ($r^2=0.89$, F=47.63, df=28, $p<0.001$; see Figure 5 for a graphical representation of the model's fit). The variables were scaled prior to analysis. Positive values of parameter estimate B indicate that SLA is increased by a given variable, while negative values indicate that SLA is decreased by the variable. SE: standard error. Significance level: *** $p< 0.001$, ** $p< 0.01$, * $p< 0.05$

Variable	B	SE	Significance level
Mean annual temperature	4.0083	0.3073	***
Mean annual temperature^2	-1.982	0.3153	***
Disturbance	-0.8201	0.2924	**
Phosphorous	0.7529	0.2946	*

The direct effects of these factors can be observed when the community-weighted SLA values of two sites, N2 (17.90 mm^2 mg^{-1}) and N27 (24. 51 mm^2 mg^{-1}), under similar climatic conditions (mean annual temperatures of 5.5 and 5.7°C, respectively) are compared to each other. The distinct difference between the SLA values can be explained by the fact that the soil phosphorous content of the N2 site was nearly four times lower than that of the N27 site (4.5 vs. 20.2 mg/kg of soil), and this difference significantly influenced the observed SLA values (compare to Ordoñez *et al*. 2009). Additionally, the influence of these factors on SLA could explain the high within-site variability of the trait values obtained at the species level (Figure 3) because their variation can create a range of microhabitats (Ackerly *et al*. 2002), especially in a heterogeneous alpine environment (Körner 2007), as discussed above.

Conclusions

The advantages of a functional trait as an indicator of the impacts of a changing environment on plant performance can only be fully realised if the trait is clearly linked to environmental gradients (Lavorel & Garnier 2002; Cornelissen et al. 2003) and all important sources of the trait variation are known (Ackerley *et al*. 2002; Vile *et al*. 2006; Albert *et al*. 2010; Lepš *et al*. 2011). The strong SLA-temperature relationship detected in the present study provides convincing evidence that this trait can be used in future studies to estimate plant responses to changing temperatures at the species and community levels. Furthermore, the described patterns of SLA variation at the population, species and community levels have three important implications for the practical application of the trait. First, the intraspecific variability of SLA values detected along the temperature gradient indicates that neglecting this source of variation by using “fixed” trait values for individual species may result in a great underestimation of the community trait composition response to environmental variability. Second, although both species turnover and changes in the plant composition within communities significantly contribute to the variation in SLA along the temperature gradient, we recommend the use of community-weighted values because they reveal more regular patterns than the underlying distribution of species values. Third, the extent of the variability in SLA along the temperature gradient is modified by the influence of other environmental factors, such as soil fertility and disturbance. Hence, these factors need to be considered when assessing the response of SLA to temperature. This situation suggests that trait values from existing trait databases, such as the world wide GLOPNET (Wright *et al*. 2004) and TRY (Kattge *et al*. 2011) or regional LEDA (Kleyer *et al*. 2008) and BioPOP (Poschlod *et al*. 2003) databases, should be used with caution, as they rarely indicate intraspecific variation or provide any climatic, habitat or edaphic information.

Chapter 3

The temperature sensitivity of pollen germination shapes species distribution patterns

Abstract: Although plant distribution patterns have been well documented, knowledge regarding the ecophysiological mechanisms that control the geographical ranges of plant species is scarce. Using the little-known method of analysing the thermal regulation of fecundation, we show here for the first time that pollen germination and tube growth are direct correlates for predicting species distribution ranges, in this case along an elevational gradient. We found a strong and positive relationship between the minimal and optimal temperatures of pollen germination and temperature conditions at our collection sites ($r^2=0.41$, $p<0.01$ and $r^2=0.2$, $p<0.05$, respectively). For pollen tube growth, the minimal and optimal temperatures of the process were also significant predictors of species occurrence under the respective climatic conditions ($r^2=0.61$, $p<0.001$ and $r^2=0.57$, $p<0.001$, respectively). The strong relationship found between the temperature requirements of the progamic phase and habitat temperature could both help in understanding the present species distribution along climatic gradients from a functional perspective and assist in better projecting future distributional range alterations caused by environmental changes.

Introduction

Temperature has been long recognised as a main factor driving plant distribution along latitudinal or elevational gradients (Salisbury 1926; Woodward & Jones 1984; Archibold 1995). Using this correlation, a large number of vegetation-climate models have been developed to illustrate plant distribution patterns at different scales (Meusel 1965; Woodward 1987; Prentice *et al.* 1992). Although these models have a high informative value, a question remains regarding which mechanisms shape the temperature distribution of species. Studies on plant persistence under low temperatures have proposed that the frost resistance of vegetative organs, such as buds or leaves and stems, could be used as a main predictor of plant distribution (Sakai 1987; Körner 1999; Larcher 2000; Taschler & Neuner 2004). However, this ecophysiological mechanism explains only a portion of large-scale distribution ranges, such as the borders of main floristic or vegetation zones, as the frost resistance of plant species from different biomes is often overlapping (Sakai 1987; Körner 1999; Larcher 2004). Additionally, the geographical limits of vegetative survival may be determined by very different environmental conditions than those at other stages of the plant life cycle (Woodward 1987; Woodward 1997; García *et al.* 2000). Therefore, an accurate understanding of how temperature shapes the distribution of a plant species, both overall and at smaller (regional and local) scales, remains one of the major challenges of modern ecology (Bykova *et al.* 2012).

In contrast to vegetative growth, reproduction processes are intimately related to ambient temperatures (Zinn, Tunc-Ozdemir & Harper 2010; Bykova *et al.* 2012) and therefore may be primarily responsible for the geographical boundaries of a species (Pigott & Huntley 1981; Pigott 1992; García *et al.* 2000; Jump & Woodward 2003). For a given species, environmental favourability declines from the core to the periphery of its area, a fact that may negatively affect the regenerative performance of a plant. Indeed, seed set progressively decreases from a maximal capacity in the centre of the area until the quality and quantity of the produced seeds are below that necessary for successful and long-term regeneration (García *et al.* 2000; Jump & Woodward 2003). Consequently, the inability to reproduce out of an ecological optimum limits the further expansion of a species in space (Grubb 1977; Woodward & Jones 1984; Pigott 1992; McKee & Richards 1996). For example, the seed production of lowland species drastically reduced with increasing elevation (Hofgaard 1993; Kullman 1993), whereas alpine species are able to complete reproduction even under the extreme climatic conditions of high elevations (Ladinig & Wagner 2005; Wagner, Steinacher & Ladinig 2010). Although the climatic control of species ranges is apparently mediated through seed production (Grubb 1977; Woodward & Jones 1984), the stage of the reproduction process, which could be understood in ecophysiological terms and could have predictive value in defining the distributional range of a species, is unknown.

Climate-induced variations in the production of seed crops may result from a specific physiological limitation of seed development (Pigott 1992; Zinn, Tunc-Ozdemir & Harper 2010). Although various stages of seed development were found to be temperature dependent (e.g., (Henttonen *et al.* 1986; Peet, Willits & Gardner 1997)), numerous experimental studies have demonstrated that, for the completion of successful fertilisation, both pollen germination (PG) and pollen tube growth (PTG) have highly specific requirements of ambient temperature (Weinbaum, Parfitt & Polito 1984; Elgersma, Stephenson & Nijs 1989; Kakani *et al.* 2005; Boavida & McCormick 2007; Steinacher & Wagner 2012). However, despite the validated dependence of fecundation success on habitat temperature, there are only a few studies that have related fecundation requirements to the geographical range of the investigated species (Pigott & Huntley 1981; Pigott & Warr 1989; Pigott 1992). For example, Pigott and Huntley (1981) have demonstrated that the temperature sensitivity of pollen tube growth and the short period of stigmoid and stylar receptivity in *Tilia cordata* could account for the northern limit of the species in the British Isles. Unfortunately, this process has not since received the attention it deserves.

Here, we explore the relationships between the temperature requirements of the progamic phase (PG and PTG) and species distributional ranges. We hypothesise that the distribution of a species along a gradient of mean annual temperature is related to the specific temperature requirements of the progamic phase. In particular, we expect that the distribution of a species with a high temperature requirement for the progamic phase is limited to the warmer part of the temperature gradient due to increasing negative temperature stress.

Materials and methods

Species selection and study area

Elevational gradients are suitable tools to investigate plant-climate interactions because there is notable evidence that the mean annual temperature (MAT) is strongly negatively correlated to elevation (0.6 K per 100 m) (Sakai 1987; Körner 1999; Kőrner 2007). Therefore, for this study, we selected 21 herbaceous species with different elevational distributions in Southern Germany (after (Oberdorfer 2001); Table 4; Appendix 4). All of the selected species occur in one vegetation type (calcareous grasslands) and share similar habitat preferences with regard to light, water, soil physics, and chemistry (Ellenberg *et al.* 1991; Oberdorfer 2001); thus, temperature is the main explanatory variable for their distributional ranges.

The pollen grains for the germination experiments were obtained from plants growing in the central part of their distribution along the elevational gradient. The pollen samples were mainly collected in the Berchtesgaden National Park (Bavaria, Germany); pollen grains from *Globularia cordifolia* L., *Primula veris* L., and *Pulsatilla vulgaris* L. were collected from a calcareous grassland located at 450 m a.s.l. (Garchinger Heide, Southern Bavaria, Germany). For every collection site, data on the mean annual temperature were obtained from the closest weather station and are presented as the mean values for the last 10 years of observation.

Pollen germination experiments

Pollen collection

To minimise the effect of intraspecific variation on pollen germination measurements (Kakani *et al.* 2005), fresh flowering buds (1-3 days before opening) were randomly collected in the field from at least 30 individual plants growing at a distance of 2-5 metres from each other. After collection, the buds were immediately transported to laboratory. Before further processing, the buds were disinfected by spraying with 96% ethanol. The anthers were then removed manually using forceps and left to dry for 2-3 days in an exsiccator filled with silica gel. To obtain the pollen grains, the dried anthers were crushed into small pieces and passed through a 200-µm sieve. Before starting the experiments, the pollen grains were stored at 5 ºC; all germination experiments were performed with pollen samples not older than 7 days.

Pollen germination experiments

The media used for the germination experiments contained nutrient salts (0.01% H_3BO_4, 1 mM $Ca(NO_3)_2$, 1 mM $CaCl_2$, and 1 mM $MgSO_4$) and different concentrations of sucrose, from 10% to 30% (Brewbaker & Kwack 1963). To reduce microbial and fungal contamination, the glassware and stock solutions of the salts were autoclaved before use; the pollen germination media were sterilised by filtration. Fresh germination media were prepared and stored at 5 °C for no longer than three days prior to use.

Preliminary tests were required due to the species-specific sensitivity of pollen to the concentration of sugar in the germination media (Bajaj 1987). To this end, hydrated pollen (see below) was mixed with germination media that varied in the sucrose content (from 10% to 30% with a 2% step) and allowed to germinate at room temperature for 18 hours. The medium with the highest germination rates and longest pollen tubes was considered as optimal and used in the germination experiments.

To avoid the bursting of the pollen grains, a hydration procedure was performed over a saturated KCl solution for 6 hours at 5 °C (Connor & Towill 1993). After hydration, the pollen was mixed with the appropriate pollen germination media, and 150 µl of the mixture was pipetted into a germination chamber that was produced by cutting a 96-well PCR plate into 24 pieces (with 4 wells each). The preprocessed pollen samples were maintained at 9 different temperatures (5, 9, 12, 16, 20, 23, 27, 31, and 34 °C) in a thermogradient table (RUMED 5990, RubarthApparate GmbH, Germany). Each temperature was replicated six times; due to the limited amount of *Globularia cordifolia* pollen, the number of replicates was reduced to four. After 18 hours, pollen germination was terminated by pipetting ≈100 µl formalin acetic alcohol (Pigott & Huntley 1981) into each germination chamber. The chambers were stored at 5 °C prior to the processing of the germinated samples.

Data analysis

Sample processing

Pollen germination was estimated by examining 300 pollen grains (approx. 10-15 microscopic fields of view) in each replicate. Germination was defined as having occurred when the length of the pollen tube was at least two times greater than the grain diameter (Kakani *et al.* 2005). The pollen germination percentage was determined by dividing the number of germinated pollen grains per field of view by the total number of pollen grains per field of view and is expressed as a percentage.

To estimate the effect of temperature on pollen tube growth, images of 25 randomly selected pollen tubes from each replicate were captured with Axiovision version 4.3. software using an AxioCamMR camera (Carl Zeiss, Germany). The pollen tube lengths were measured manually using ImageJ software (Abramoff, Magalhães & Ram 2004).

Cardinal temperatures

Data on the pollen germination rates and pollen tube length were analysed using linear and nonlinear regression techniques to quantify the responses to temperature. Quadratic, cubic, and bilinear equations were applied to the data and examined to determine the best-fit model (Kakani *et al.* 2005). The quadratic equation (Equation 1) provided the greatest r^2 values and smallest root mean squared deviation for both PG and PTG.

The following equations were used to estimate the cardinal temperatures, minimum ($T_{\min}$), optimum (T_{opt}), and maximum ($T_{\max}$), for PG and PTG of all of the species examined (Equations 2, 3, and 4). Linear models (R software (R Development Core Team 2012)) were used to estimate the constants in the quadratic equation.

Pollen germination (PG, %) or pollen tube growth (PT)=

$$\text{PG(\%) or PT} = a + bT + cT^2 \qquad (1)$$

$$T_{opt} = -\frac{b}{2c} \qquad (2)$$

$$T_{\min} = T_{opt} - \frac{\sqrt{b - 4ac^2}}{2c} \qquad (3)$$

$$T_{\max} = T_{opt} + \frac{\sqrt{b - 4ac^2}}{2c} \qquad (4)$$

where a, b, and c are the equation constants, T is the temperature at which germination and tube growth were studied, and $T_{\min}$, T_{opt}, and $T_{\max}$ are the minimum, optimum, and maximum temperatures for pollen germination and pollen tube length.

Relationship between PG/PTG and MAT

To estimate the relationship between habitat temperatures and the temperature requirements of the progamic phase, the calculated cardinal temperatures were associated with the MATs of the collection sites using linear models. MAT was selected as an aggregated indicator of the temperature conditions of the collection site because elevational gradients encompass multiple variations (e.g., annual temperatures, length of the vegetation period, number of frost days, growing–degree days) that autocorrelate with each other (Kőrner 2007). In all of the models, nonlinear terms were also considered ($T_{cardinal}$ ~ MAT + MAT^2), and the model assumptions (normal distribution of residual errors and homogeneous variances) were met in all cases.

Prior to estimating the relationship between the cardinal temperatures of PG and PTG and MAT, we calculated an estimate of *K*, which is a parameter for a phylogenetic correlation within a trait that ranges from zero to one (Blomberg, Garland & Ives 2003). Values not significantly different from 0 suggest no phylogenetic signal in a trait, whereas *K* values > 1 indicate a strong phylogenetic signal and conservatism of the trait (Krasnov, Poulin & Mouillot 2011). The *K* value calculations were based on a dated phylogeny of a large European flora (Durka & Michalski 2012) and implemented in the R statistical environment (package *picante*) (Kembel *et al.* 2010; R Development Core Team 2012).

Results

Cardinal temperatures of pollen germination

Due to the presence of dried anthers in the sample, the pollen germination rates were not obtained for *Carex flacca*. The pollen germination rates ranged from 11.8% for *Lychnis flos-cuculi* to 56% for *Pulsatilla vulgaris*, with an average of 31.7% (Table 4). The cardinal temperatures for PG differed greatly among the species examined. The values of $T_{\min}$ ranged from –5.6 °C (*Helleborus niger*) to 12.8 °C (*Ranunculus nemorosus*), with an average of 2.6 °C. The optimum temperature (T_{opt}) ranged from 15.7 °C (*Helleborus niger*) to 33.9 °C (*Ranunculus nemorosus*), with an average of 22.2 °C. The $T_{\max}$ values varied from 33.7 °C for *Soldanella alpina* to 55 °C for *Ranunculus nemorosus*, with an average of 41.8 °C (Table 4).

Cardinal temperatures of pollen tube growth

Similar to PG, the species differed considerably with regard to pollen tube length at the minimum, optimum, and maximum cardinal temperatures. The pollen tube length ranged from 173 µm for *Primula minima* to 1280 µm for *Lychnis flos-cuculi*, with an average of 662.5 µm (Table 4). The values of $T_{\min}$ ranged from –5.9 °C (*Phyteuma orbiculare*) to 11.4 °C (*Ranunculus nemorosus*). The magnitude of T_{opt} ranged from 17 °C (*Campanula alpina*) to 28°C (*Globularia cordifolia*). The $T_{\max}$values ranged from 35.2 °C for

Campanula alpina to 48.4 °C for *Carex flacca*. The mean values for $T_{\min}$, T_{opt}, and $T_{\max}$ were 3.1, 21.9, and 40.8 °C, respectively. For visualisation, the response of PTG to the temperature treatments of three species (*Carex flacca, Soldanella alpina*, and *Gentianella aspera*) from climatically contrasting habitats are shown in Figure 6.

Phylogenetic signal

A phylogenetic signal was not detected in pollen germination, irrespective of the cardinal temperature, indicating a lack of association of the process with phylogeny ($T_{\min}$ (K=0.08, p=0.78); T_{opt} (K=0.08, p=0.81); $T_{\max}$ (K=0.15, p=0.64)). The low and not significant K values calculated for the pollen tube length indicate that this trait is similarly not phylogenetically constrained ($T_{\min}$ (K=0.15, p=0.52); T_{opt} (K=0.2, p=0.4); $T_{\max}$ (K=0.36, p=0.07)).

Correlations between cardinal temperatures and habitat temperatures

The results of the linear models associating the cardinal temperatures of the progamic phase and the habitat temperatures are represented in Figure 7.

We found that $T_{\min}$ of PG was significantly correlated with MAT (Figure 7a, r^2=0.41, F=12.59, df=18, p=0.002). The relationship between T_{opt} and MAT was also significant (Figure 7c, $r^2 = 0.2$, F=4.5, df=18, p=0.048).

An analysis of the data for PTG showed that the minimal and optimal temperatures were significant predictors of the occurrence of the species along the MAT gradient (Figure 7b, $r^2=0.61$, $F=27.9$, $df=19$, $p<0.001$ and Figure 7d, $r^2=0.57$, $F=24.3$, $df=19$, $p<0.001$, respectively).

In contrast, the maximal temperatures of PG and PTG did not show any statistically significant correlations with MAT (Figure 7e, $r^2=0.02$, $F=0.44$, $df=18$, $p=0.8$ and Figure 7f, $r^2=0.12$, $F=2.4$, $df=19$, $p=0.12$, respectively). In all cases, the nature of the correlation between the cardinal temperatures and MAT had a linear character.

Table 4. Cardinal temperatures of pollen germination and pollen tube growth for the investigated species. *Tmin* - initial temperature, *Topt* - optimal temperature, *Tmax* - maximal temperature.

Species	**Species distribution ranges**		**MAT of collection site, °C**	**Pollen germination**				**Pollen tube length**			
				Maximum germination rate, %	**Cardinal temperatures, Cº**			**Maximum pollen tube length, µm**	**Cardinal temperatures, Cº**		
	Elevation, m a.s.l	**MAT, °C**			**Tmin**	**Topt**	**Tmax**		**Tmin**	**Topt**	**Tmax**
Anemone nemorosa L.	300 - 1700	3.9 - 9.0	6.06	20	6.5	26.2	45.9	510	3.9	24.7	45.5
Caltha palustris L.	300 - 1400	4.9 - 9.0	6.82	45.9	9.1	21.9	34.8	1129	9.8	23.1	36.5
Campanula alpina Jacq.	1800 - 2300	1.9 - 3.6	2.74	34.5	-4.1	15.7	35.6	412	-1.2	17	35.2
Campanula scheuchzeri Vill.	1000 - 2200	2.2 - 6.2	3.54	32	-3.7	16.8	37.3	526	-4.8	17.4	39.5
Carex flacca Schreber	300 - 1400	4.9 - 9.0	6.25	-	-	-	-	855	1.4	24.9	48.4
Gentiana asclepiadea L.	600 - 1800	3.4 - 7.5	3.66	19	-1.7	20.1	41.9	397	-2.4	20.3	43.1
Gentiana aspera Hegetschw.	1800 - 2300	1.9 - 3.6	2.91	16.4	3.4	26.1	48.8	758	-3.7	17.1	37.9
Gentiana pannonica Scop.	1300 - 2100	2.6 - 5.6	5.12	12.4	3.9	27.1	50.4	850	2.1	19.5	36.9
Globularia cordifolia L.	300 -1400	4.9 - 9.0	8.03	46.6	7.8	29.2	50.6	733	9	28	47
Helleborus niger L.	600 - 1400	4.9 - 7.5	6.34	14.8	-5.6	15.7	37	309	0.6	18.8	37.1

Table 4. Cardinal temperatures of pollen germination and pollen tube growth for the investigated species. *Tmin* - initial temperature, *Topt* - optimal temperature, *Tmax* - maximal temperature (continued).

Species	Species distribution ranges		MAT of collection site, °C	Pollen germination				Pollen tube length			
				Maximum germination rate, %	Cardinal temperatures, C^{o}			Maximum pollen tube length, µm	Cardinal temperatures, C^{o}		
	Elevation, m a.s.l	MAT, °C			Tmin	Topt	Tmax		Tmin	Topt	Tmax
Lychnis flos-cuculi L.	300 - 1700	3.9 - 9.0	6.06	11.8	6.1	20.7	35.3	1280	6.4	21.4	36.3
Phyteuma orbiculare L.	1400 - 2200	2.3 - 4.9	3.54	33	-4.2	18.8	41.8	394	-5.9	17.6	41.1
Plantago lanceolata L.	300 - 1400	4.9 - 9.0	6.06	30.7	3.1	22.3	41.4	1025	5.9	25.3	44.8
Primula minima L.	1800 - 2300	1.9 - 3.6	2.74	44	-1.5	16.3	34.2	173	1	19.6	38.1
Primula veris L.	300 - 1000	6.2 - 9.0	8.03	37	8	22.6	37.3	704	8.6	23.6	38.6
Pulsatilla vulgaris Mill.	300 - 1000	6.2 - 9.0	8.03	56	6.5	22.1	37.6	347	8.2	26.6	44.9
Ranunculus montanus Willd.	1300 - 2200	2.3 - 5.2	4.23	26.8	2.9	25.6	48.4	535	3	24.5	45.9
Ranunculus nemorosus DC.	300 - 1100	5.9 - 9.0	6.34	14.2	12.8	33.9	55	733	11.4	25.9	40.4
Ranunculus polyanthemophyllus W. Koch & Hess	300 - 1100	5.9 - 9.0	6.74	48	2.6	23.8	45.1	880	4.6	21.7	38.9
Soldanella alpina L.	1000 - 2300	1.9 - 6.2	4.39	49.4	2.1	17.9	33.7	680	4.4	20.1	35.8
Trollius europaeus L.	1300 - 2100	2.6 - 5.6	5.4	41.3	-2.2	21.3	44.8	682	2.7	24	45.3

Discussion

The results of our study show that the minimal and optimal temperatures for both pollen germination and pollen tube growth are strongly negatively correlated to the mean annual temperature of the habitat (r^2=0.41, r^2=0.2, r^2=0.61, and r^2=0.57, respectively). These results confirm our hypothesis that the distribution of species along a MAT gradient is related to the specific temperature requirements of the progamic phase. Due to favourable climatic conditions, the pollen grains of species from habitats with a higher MAT are adapted to germinate and grow under relatively high temperatures (Weinbaum, Parfitt & Polito 1984; Jakobsen & Martens 1994; Pasonen, Käpylä & Pulkkinen 2000; Kremer & Jemrić 2006). Our results are consistent with this previously found pattern, as the pollen of all the tested species (with the exception of *Helleborus niger*) began to germinate and grow at relatively high temperatures. A decreasing MAT along a climatic gradient is coupled to the increasing probability of negative temperature stress in the form of freeze-thawing cycles in spring or autumn or as freezing episodes during the growing season (Sakai 1987; Körner 1999). Species of cold habitats can adapt fecundation to these changes by reducing their temperature requirements for the progamic phase (Zamir, Tanksley & Jones 1981; Steinacher & Wagner 2012). These results of our experiments confirm this: the PG and PTG cardinal temperatures of species from relatively cold habitats (MAT < 5.4 °C) did not exceed 5 °C (Table 4).

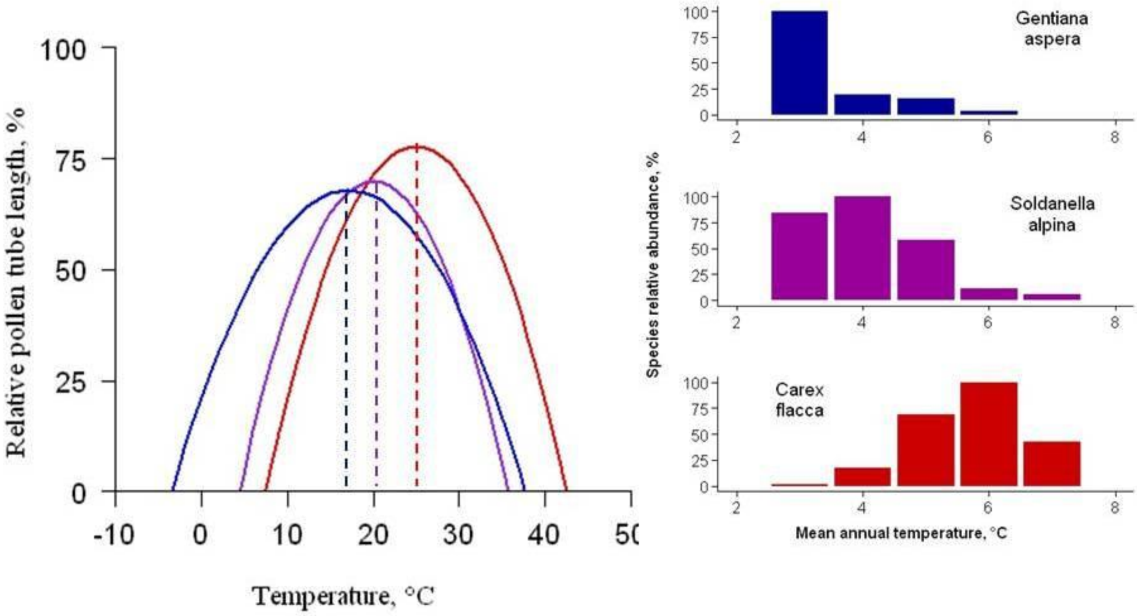


Figure 6. Differences in the temperature requirements for pollen tube growth of three species (*Carex flacca*, *Soldanella alpina*, and *Gentiana aspera*) occurring in climatically contrasting habitats. The cardinal temperatures were estimated by fitting the quadratic function to the experimental data for pollen tube length (see Materials and Methods section). The species relative abundance along the gradient of mean annual temperatures was estimated based on 3000 vegetation surveys performed in the Berchtesgaden National Park.

The strong correlation between the habitat temperature and progamic phase suggest that the temperature requirements of PG and PTG may help to explain species distribution patterns under the respective climatic conditions. The pollen germination and pollen tube growth of species from warm habitats require relatively high temperatures to successfully complete fecundation. Therefore, a decreasing annual temperature along a climatic gradient will negatively affect the reproduction processes of the respective species due to the increasing mismatch between ambient and temperature required for fecundation. Hence, even though vegetative growth is not limited by temperature, a reduction in the reproduction inputs into the plant life cycle will affect the distribution of the species because they can neither further expand their range nor maintain the long-term existing populations within the geographical limits (Grubb 1977; Pigott & Huntley 1981; Turnbull, Crawley & Rees 2000). In contrast, species with lower requirements of temperature for PG and PTG can follow the increasing negative temperature stress and successfully complete fecundation, even in regions with a low MAT. This fact allows such species to extend their ranges northward or to the alpine zone. However, such factors as a low competitive ability due to a low growth rate and short stature (Woodward 1975; Körner 1999) may limit their expansion into habitats in regions of a higher MAT.

The results presented here suggest that this finding will help reach a better understanding of the existing patterns of plant species distribution and in improving the accuracy and specificity of predictions of vegetation shifts under global change scenarios. In the last decade, a strong focus has been placed on the modelling of vegetation-climate interactions. However, these models still suffer from an essential lack of temperature-specific ecological data and a mechanistic understanding of how environmental factors shape current species distributions.

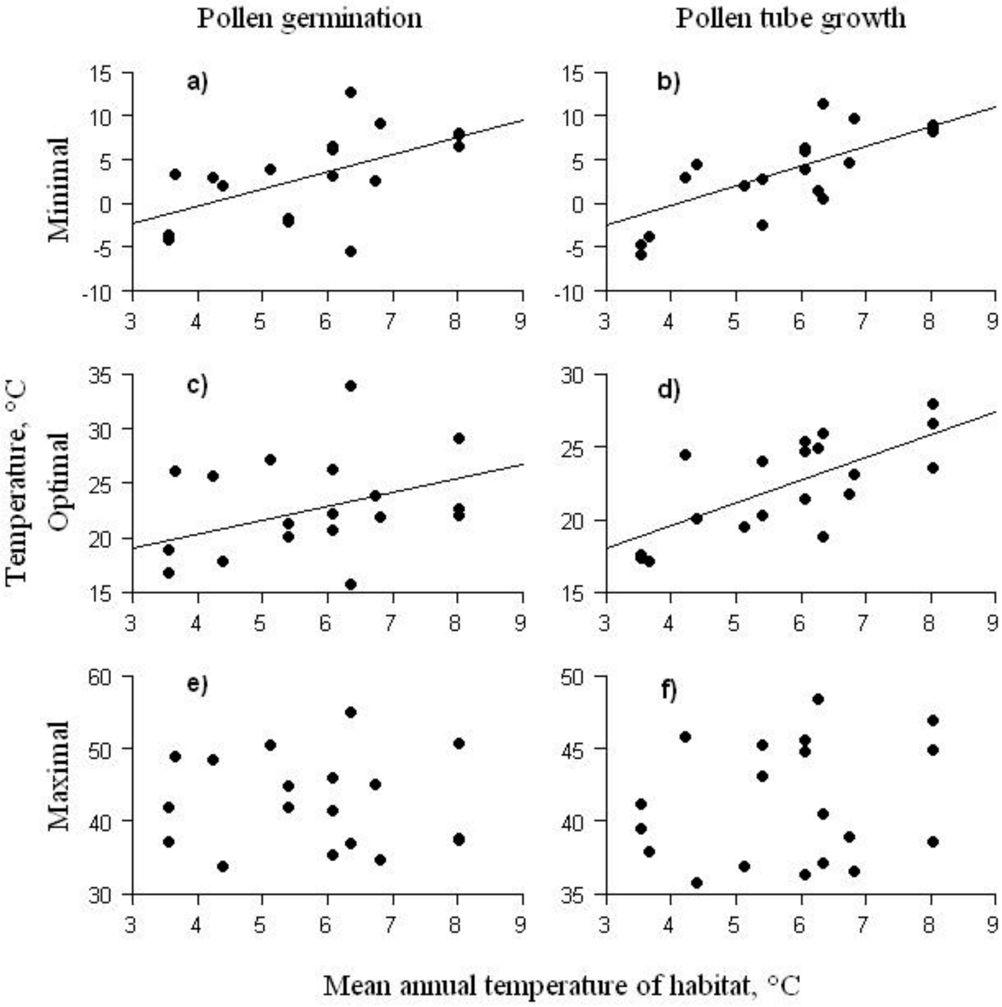


Figure 7. Relationships between the temperature requirements of fecundation and the temperature conditions of habitats where species occur. a, b, Minimal temperature of pollen germination and pollen tube growth. c, d, Optimal temperature of pollen germination and pollen tube growth. e, f, Maximal temperature of pollen germination and pollen tube growth.

The temperature requirements of PG and PTG could easily be integrated into models as they can quantitatively estimate the success of reproduction under certain climatic conditions and are technically easy to obtain (in contrast to other stages of the plant life cycle).

Chapter 4

Initial temperature of seed germination as related to species occurrence along a temperature gradient

Abstract

Reproductive stages of life cycle are important for the explanation of distribution patterns of plant species at different scales, due to their extreme vulnerability to environmental conditions. Despite reported evidences that seed germination is related to habitat macroclimatic characteristics, such as mean annual temperature (MAT) and precipitation, the role of this trait in controlling plant species distribution was never systematically and quantitatively evaluated. Using the data on seed germination along temperature gradient for 49 species originating from contrasting climatic conditions, we show here that initial temperature of seed germination (T_{min}) is direct correlate for predicting species distribution ranges along temperature gradient. Our study reveals that T_{min} is strongly negatively correlated to habitat temperature; among the studied species T_{min} clearly increased with decreasing MAT ($r^2 = 0.57$, $p<0.001$). Considering phylogenetic biases, coevolution of seed traits as well as precipitation along with microclimatic factors did not affect the

strength of this relationship. Our results suggest that the T_{min}-MAT relation can particularly provide insights into species distribution patterns, vegetation dynamics and community assembly rules along altitudinal and latitudinal gradients. We argue that including the T_{min} in species distribution models may help to improve the accuracy and specificity of predictions of vegetation shifts under global change scenarios.

Introduction

Plant functional traits (PFT) play an important role in modern ecology as universal tools to understand mechanisms behind vegetation patterns at global to local scales (Diaz, Cabido & Casanoves 1998; Weiher *et al.* 1999; Lavorel & Garnier 2002; Canadell *et al.* 2007; Pérez-Harguindeguy *et al.* 2013). In the last decades a big effort has been done to approve the relevance of the PFTs to explain species distribution patterns and community assembly rules. Dozens of plant traits were found to be related to temperature (De Frenne *et al.* 2013), water availability (Sperry & Hacke 2002), soil nutrients (Hodgson *et al.* 2011), disturbance (Pausas *et al.* 2004) and many others (for detailed review see (Cornelissen *et al.* 2003)). Despite this, even a short glimpse at the vast literature on the PFTs shows that mainly vegetative traits (whole plant, leave and stem traits) are considered and regenerative traits often remain underrepresented (Kleyer *et al.* 2008; Kattge *et al.* 2011; Pérez-Harguindeguy *et al.* 2013).

However, recent studies demonstrate that reproductive stages of plant life cycle such as fecundation, seed germination and seedling recruitment are essential to understand distribution of plants across difference scales, due to their extreme vulnerability (Grubb 1977; Jump & Woodward 2003; Fenner & Thompson 2005; Bykova *et al.* 2012; Poschlod *et al.* 2013). Therefore, in order to fully reveal processes controlling species distribution and abundance, more research on regeneration processes is urgently needed (Walck *et al.* 2011; Bykova *et al.* 2012; Poschlod *et al.* 2013).

Although climate, with temperature being the most important component, has a strong influence on plant recruitment (Grubb 1977; Baskin & Baskin 1988; Grime 2002; Walck *et al.* 2011), aspects of seed ecology are still rarely considered for the explanation of distribution patterns of plant species (Poschlod *et al.* 2013). It is suggested that species occurrence along climatic gradients is related to both dormancy depth and germination requirements of non-dormant seeds (Baskin & Baskin 1998; Fenner & Thompson 2005; Poschlod *et al.* 2013). Dormancy is well known as a good predictor of species occurrence along temperature and precipitation gradient; the longer seeds are exposed to environmental conditions unsuitable for germination such as cold or dry periods, the deeper dormancy is (Allen & Meyer 1998; Baskin & Baskin 1998; Schwienbacher *et al.* 2011; Walck *et al.* 2011). Although the limiting role of local environmental factors (e.g. light, temperature fluctuations, soil surface, etc.) in community assembly has been exhaustively investigated (e.g. (Grime *et al.* 1981; Baskin & Baskin 1998)), less is known, in contrast to dormancy, how germination requirements of non-dormant seeds are related to macroclimatic habitat characteristics such as mean annual temperature (MAT) and precipitation. As it is already documented in some cases, these requirements may be habitat specific (Thompson 1970b; Grime *et al.* 1981; Trudgill, Squire & Thompson 2000). For example, species of warm habitats begun to germinate immediately after snow melting, whereas germination of species from cold habitats (e.g. high latitudes and altitudes) were triggered by temperatures only above 10°C (Billings & Mooney 1968; Thompson 1968). However, despite the previously proposed link between germination requirements and macroclimatic environmental factors (Thompson 1970a), it has never been systematically and quantitatively evaluated.

Here, we explore to which extent germination requirements of species with different distributional ranges are related to their macroclimatic habitat characteristics. More specifically, we test the hypothesis that initial temperature of seed germination (hereafter T_{min}) is a good predictor of species occurrence along a gradient of MAT. Moreover, due to complexity of seed germination patterns (Baskin & Baskin 1998; Fenner & Thompson 2005), we considered in our study three biases which could hamper the mechanistic understanding of T_{min} variation along the temperature gradient:

1) Phylogenetic bias. Since related species are likely to share similar attributes (Harvey & Pagel 1991), variations in $T_{\min}$ as other seed traits (Baskin & Baskin 1988) may be phylogenetically constrained. For example, grasses tend to germinate under lower temperatures (Grime *et al.* 1981), whereas seeds of *Cyperaceae* do not begin to germinate if ambient temperature is below 10 °C (Schütz & Rave 1999);
2) Effect of other than temperature environmental factors. Precipitation, which is another important macroclimatic environmental factor for seedling recruitment, may significantly modify seed germination response to ambient temperature. For example, seed of species from habitats with high level of water supply tend to germinate under relatively high temperatures (Grime *et al.* 1981), whereas seeds from habitats with distinct dry periods (e.g. Mediterranean) (Thompson 1970d; Bell *et al.* 1995) germinate just above freezing temperatures, which coincide with wet seasons. Additionally, microclimate may modify or even neglect the role of habitat temperature as germination cue, due to the fact that seed germination is primarily influenced by local climatic conditions (Grubb 1977; Schütz & Rave 1999). For example, wetland species prefer to germinate rather under relatively high temperatures (Grime *et al.* 1981; Schütz & Rave 1999).

3) Coevolution of traits. Seed morphological traits and germination patterns may be coevolved (Jurado & Westoby 1992; Bond, Honig & Maze 1999; Milberg, Andersson & Thompson 2000), suggesting that T_{min} could also vary, regardless ambient temperatures, e.g. among seeds with different size (Grime *et al.* 1981; Schütz & Rave 1999) and different thickness of seed coat (Gardarin *et al.* 2010).

Therefore, the relative effects of precipitation, species' habitat characteristics (light, soil moisture, soil fertility), seed traits (seed weight and seed coat thickness) as well as species phylogenetic affiliation on T_{min}- MAT relation were estimated as well.

Materials and methods

Temperature gradient, species selection and seed collection

We selected an altitudinal gradient as a suitable tool for estimating the T_{min} variations because there is clear evidence that MAT is strongly negatively correlated to altitudinal elevation (0.6K per 100 m or shortening of the vegetation period by 3-5 days per 100 m) (Sakai 1987; Diaz & Cabido 1997; Körner 1999; Kőrner 2007). Therefore, for the study we selected 95 plant herbaceous species with different altitudinal distribution in Southern Germany (Oberdorfer 2001); Appendix 5) and subsequently collected seeds of these species. All selected species occur in one vegetation type (calcareous grasslands) and share similar habitat preferences in light, water, soil physics and chemistry (Ellenberg *et al.* 1991; Oberdorfer 2001), so that temperature is the main explanatory variable for their distributional ranges.

Seeds were mainly collected in the Berchtesgaden National Park (Bavaria, Germany), which is situated in the Bavarian Alps (northern part of the Limestone Alps) and is approximately 200 km² in size. The relief of the National Park can be characterised as typically alpine, with steep mountain peaks composed of Triassic lime and dolomite rocks (Marke *et al.* 2013). The climate in the region is a typical mountain climate, showing a large decrease in mean annual air temperatures from +7° to –2°C (from 603 to 2713 m above sea level (a. s. l.), respectively). The mean annual precipitation varies from year to year, ranging from approximately 1500 to 2600 mm per year (Marke *et al.* 2013). Seeds of seven species (*Achillea millefolium*, *Buphthalmum salicifoliu*, *Carlina acaulis*, *Cynosurus cristatus*, *Hypochaeris radicata*, *Pimpinella major* and *Silene dioica*) were collected from natural populations, which were located close but outside the National Park. For every collection site data on MAT and precipitation were obtained from the National Park weather stations, which were located at the altitudes closest to the collection sites. The data on MAT are presented as mean values for the last 10 years of observation.

Fully ripened seed and fruits (in the following named “seeds”) of the selected species were collected at the end of summer and in the autumn of three study years (2009-2011) from populations located in the middle part of species‘ altitudinal distribution (i.e. under optimal ecological conditions). In the field seeds were collected from randomly chosen individuals (>50 individuals per species) in a relatively big area (500 – 1000 m²) and thoroughly mixed. After collection, seeds were air-dried for several days, cleaned and, following to the recommendations of Baskin & Baskin (1998), kept dry in a cold room under 4 °C until the beginning of the germination experiments.

Dormancy breaking and optimal conditions for germination

Before starting the seed germination experiments along temperature gradient (see below), optimal dormancy-breaking conditions for every tested species were identified. To achieve that, the seeds were previously subjected to different treatments in an attempt to facilitate germination. Three main treatments were adopted (dry cold storage for 12 weeks, wet chilling for 6 and 12 weeks), and these were applied in a series, progression through which depended upon continued failure to induce germination to a high percentage (80-100%). The dry cold storage and the wet chilling were conducted in a climate chamber under a +4° C temperature regime. Since diurnal temperature fluctuations may affect germination rates of seeds in many species (Thompson, Grime & Mason 1977), their effect, additionally to the dormancy-breaking conditions, was tested as well. In order to test this effect, the seeds in Petri dishes (5 dishes, 20 seeds each) were placed in climatic chambers RUMED 1301 (RubarthApparate

GmbH, Germany) either at constant (22°C) or diurnal fluctuating temperatures (22/14° C) and 14 h light supplied by white fluorescent tubes (3800 Lux), after a dormancy-breaking treatment was applied. After incubation of the seeds in the germination chambers for 4 weeks, the germination rates from all treatments were compared and the treatment with the highest germination rates was considered to be optimal. Species, whose germination rates after all treatments were still lower than 80% (46 species), were not considered for further experiments (Appendix 5). Only species showing high germinability and rapid rates of germination in the initial tests (greater than 80% after 28 days incubation; (Grime *et al.* 1981)) were considered for further experiments.

Germination experiments along temperature gradient

In order to estimate T_{min}, seeds of 49 species (Appendix 6) were germinated along temperature gradient created in six separate climate chambers RUMED 1301 (RubarthApparate GmbH, Germany). Since fluctuating temperatures significantly increased germination rates of all tested species (data are not shown), the following temperature regime (light/dark, 14/8 hours) were used in the experiment: 10/2, 14/6, 18/10, 22/14, 26/18 and 30/22°C. All seeds were exposed to optimal dormancy-breaking conditions (see above) prior to germination tests along the temperature gradient. All germination experiments along the temperature gradient were carried out with fluctuating temperatures as this mode was found in the preliminary experiments to be the most favorable for germination of all species. For every tested temperature 8 Petri dishes (= replicates) with 25 seeds of a species were placed in a climate chamber. Due to limited number of seeds *of Allium montanum*, number of replicates as well as number of seeds were limited to 5 and 20, respectively.

Number of germinated seeds was scored every 3rd day for the first two weeks, thereafter scoring was carried out once per week until four weeks had elapsed, when the experiment was concluded. Germination was defined as the emergence of the radicle or cotyledons through the seed coat. At the end of incubation period the viability of the remaining seeds were estimated by crushing them; seeds with white, hard embryos were considered to be alive (Baskin & Baskin 1998).

Seed morphological traits

In order to measure seed coat thickness, 10 replicate seeds of each species were first exposed to X-rays at a radiation 18 kV for 10 s (Faxitron MX-20 cabinet X-ray system). Following that, the thickness of seed coat, including the integument, the fruit coat (pericarp) and, where possible, the remnants of floral pieces (Gardarin *et al.* 2010) was measured manually with the help of image analysis software (ImageJ; (Abramoff, Magalhães & Ram 2004)).

Seed mass was obtained by weighing randomly taken 100 seeds and calculating the mean weight for one single seed.

Data processing

Initial temperature of germination

In order to make the experimental data on T_{min} of germination and MAT of habitats comparable, we transformed tested temperatures, which were fluctuating, to constant ones: 10/2, 14/6, 18/10, 22/14, 26/18 and 30/22°C to 7, 11, 15, 19, 23 and 27 °C, respectively. To estimate the T_{min} values, Weibull function was fitted to the cumulative germination rates obtained from all tested temperatures at the 28th day after the experiments started. The Weibull function was selected as the most suitable for describing cumulative germination as it provides a consistently close fit to the data and, therefore, could also be used in the calculation of cardinal temperatures, i.e. initial, optimal and maximal temperatures of a process (Brown & Mayer 1988; Dumur, Pilbeam & Cragon 1990; Ritz 2010). T_{min} was considered as a temperature under which first 5 % of all seeds germinated. The curve-fit as well as estimation of the T_{min} were carried out with the help of *drc* package (Ritz & Streibig 2005) for R software (R Development Core Team 2012).

Phylogenetic signal in the trait variation

Prior to estimate the variation of T_{min} along the gradient of MAT, we calculated an estimate of *K*, which is a parameter for phylogenetic correlation within a trait that ranges from zero to one (Blomberg, Garland & Ives 2003). Values not significantly different from 0 suggest no phylogenetic signal in a trait, whereas *K* values > 1 indicate strong phylogenetic signal and conservatism of the trait (Krasnov, Poulin & Mouillot 2011). An alternative estimate of phylogenetic signal, Moran's I, which provides an indication of how phylogenetic signature changes across the phylogeny (Gittleman & Kot 1990), was also estimated. The resulting values of the later analysis do not offer any quantitative interpretation of the phylogenetic signal, because the expected value of the statistic under the assumed model is unknown a priori. However, stronger deviations from zero indicate stronger relationships between trait values and the phylogeny (Münkemüller *et al.* 2012). The phylogenetic autocorrelation in the data were estimated at four taxonomic levels: class, subclass, order and family. Additionally to these levels, we tested for phylogenetic signals within families with more than 4 species in our data set (*Asteraceae*, *Poaceae* and *Caryophyllaceae*; arbitrary level "family group"); families with less than 4 species in this case were analyzed all together (arbitrary group "other families").

Calculations of the *K* value were based on a dated phylogeny of a large European flora (Durka & Michalski 2012) and implemented in R statistical environment (packages *picante*) (Kembel *et al.* 2010; R Development Core Team 2012). The presence of phylogenetic signal in the seed traits (weight and seed coat thickness) was estimated as well by the procedure described above.

Relationship between $T_{\min}$ and temperature

To estimate the variation of the trait along the temperature gradient, we set up a full linear model. As explanatory variables for $T_{\min}$ we included MAT and annual precipitation as both simple and quadratic terms, because a trait-environment relationship can be non-linear. To test for possible effect of other environmental factors on the $T_{\min}$ – MAT relation, we also included in the model species' habitat characteristics expressed as Ellenberg indicator values (EIV) for light, moisture and soil fertility. The indicator values can help to discriminate species which may exhibit traits not strictly related to the gradient of MAT (e.g. microhabitat moisture or light peculiarities instead of temperature effects)(Zhu *et al.* 2010). Although the EIVs reflect ecological behavior of adult plants, their relevance to explain seed germination patterns has been already proven (Jankowska-Blaszczuk & Daws 2007). Since the test for phylogenetic signal detected differences in $T_{\min}$ values among big families in the data set (see Results section), relation to the family group (*Asteraceae, Poaceae, Caryophyllaceae* or arbitrary level "family group") were included in the model as interaction with MAT as well. Effect of coevolution of germination and morphological traits on $T_{\min}$ - MAT relation were also considered by including seed weight and seed coat thickness in the model. All explanatory variables were considered not to be collinear, because they were correlated weakly with each other (Appendix 7).

To meet the preconditions of the linear models (i.e., normal distribution of the residuals and homogeneity of variances), T_{min} values were log-transformed. Additionally, in order to provide estimates comparability (and herewith their relative impact), MAT, precipitation and all Ellenberg values included in the model were scaled to zero mean unit variance prior to analyses. The full model was reduced via backward selection of the least significant variables until we received the minimal adequate model (Crawley 2007). All statistical calculations were performed with the open source software R version 2.14.0 (R Development Core Team, 2012).

Results

Initial temperature of germination

We observed clear differences in species T_{min} values among tested species; values ranged from 3.3°C (*Pimpinella major*) to 14.7°C (*Carex flava*) (Table 5). To demonstrate the differences in T_{min} among species originating from different parts of the temperature gradient, final germination rates together with fitted curves of several species are shown on the Figure 8.

Table 5. Characteristics of the studied species. MAT – mean annual temperatures, EIV – Ellenberg indicator values, T_{min} - initial temperature of germination, x – indifferent.

Species	Family	Habitat characteristics		Species characterstics						
				EIV			Germinaiton traits		Seed traits	
		MAT, °C	Precipitation, mm	Light	Moisture	Soil fertility	Maximal germinaiton rate, %	Tmin	Weight, mg	Coat thickness, µm
Achillea atrata	Asteraceae	3.6	1503	9	5	3	100	10.0	0.296	35.8
Achillea clavennae	Asteraceae	3.6	1503	8	5	3	100	8.4	0.366	28.7
Achillea millefolium	Asteraceae	8.4	800	8	4	5	100	6.5	0.131	34.0
Adenostyles alliariae	Asteraceae	4.4	1631	6	6	8	100	6.0	0.401	41.5
Allium montanum	Liliaceae	3.6	1503	9	2	2	100	6.7	2.147	30.5
Anthoxanthum alpinum	Poaceae	2.9	1503	7	6	2	96	5.9	0.615	122.4
Anthoxanthum odoratum	Poaceae	6.0	1748	x	-	x	100	4.0	0.543	67.1
Arrhenatherum elatius	Poaceae	7.2	1612	8	-	7	100	5.5	3.282	261.7
Briza media	Poaceae	5.2	1631	8	-	2	100	4.6	0.404	33.1
Buphthalmum salicifolium	Asteraceae	8.2	800	8	4	3	100	4.5	0.833	104.5
Carduus defloratus	Asteraceae	2.9	1503	7	-	4	100	9.8	2.742	97.2
Carex firma	Cyperaceae	2.9	1503	9	4	2	100	10.6	0.612	38.6
Carex flava	Cyperaceae	4.9	1631	8	9	2	86	14.7	0.686	71.9
Carlina acaulis	Asteraceae	8.2	800	9	4	2	100	4.5	3.964	37.6
Centaurea jacea	Asteraceae	6.9	1748	7	x	x	100	4.4	1.72	85.8

Table 5. Characteristics of the studied species (continued).

Species	Family	Habitat characteristics		Species characterstics						
				EIV			Germinaiton traits		Seed traits	
		MAT, °C	Precipitation, mm	Light	Moisture	Soil fertility	Maximal germinaiton rate, %	Tmin	Weight, mg	Coat thickness, µm
Crepis biennis	Asteraceae	7.2	1612	7	6	6	100	3.9	0.928	42.0
Cynosurus cristatus	Poaceae	8.2	800	8	5	4	100	4.9	0.317	45.9
Deschampsia cespitosa	Poaceae	5.7	1503	6	-	3	100	5.2	0.303	22.6
Dryas octopetala	Rosaceae	2.9	1503	9	4	4	100	10.4	0.376	43.0
Erigeron glabratus	Asteraceae	3.6	1503	9	5	3	100	6.8	0.203	31.9
Geum rivale	Rosaceae	7.2	1612	6	-	4	100	9.6	1.066	49.2
Globularia cordifolia	Globulariaceae	5.9	1748	9	4	2	100	6.8	0.467	27.3
Globularia nudicaulis	Globulariaceae	4.4	1631	8	2	2	100	10.3	0.616	49.2
Horminum pyrenaicum	Lamiaceae	4.1	1631	9	5	2	100	9.5	0.617	46.5
Hypochaeris radicata	Asteraceae	8.2	800	8	5	3	100	5.4	0.695	32.4
Leontodon hispidus	Asteraceae	6.0	1748	8	5	6	100	10.1	1.68	45.9
Leucanthemum halleri	Asteraceae	3.6	1503	9	5	2	100	10.3	0.539	31.7
Leucanthemum ircutianum	Asteraceae	7.2	1612	7	4	3	100	5.8	0.396	33.6
Luzula campestris	Juncaceae	4.2	1631	7	4	3	100	7.0	0.8	-
Luzula sylvatica	Juncaceae	5.2	1631	4	5	4	100	6.3	0.824	26.2
Origanum vulgare	Lamiaceae	4.9	1631	7	3	3	100	4.3	0.063	24.4
Pimpinella major	Apiaceae	8.2	800	7	5	6	100	3.3	1.111	28.3
Plantago lanceolata	Plantaginaceae	7.2	1612	6	-	x	100	5.7	1.832	297.7

Table 5. Characteristics of the studied species (continued).

		Habitat characteristics		**Species characterstics**						
				EIV			**Germinaiton traits**		**Seed traits**	
Species	**Family**	**MAT, °C**	**Precipitation, mm**	**Light**	**Moisture**	**Soil fertility**	**Maximal germinaiton rate, %**	**Tmin**	**Weight, mg**	**Coat thickness, µm**
Poa alpina	Poaceae	3.6	1503	7	5	7	100	5.4	0.342	26.5
Primula minima	Primulaceae	2.9	1503	8	5	1	88	11.2	0.1	84.5
Ranunculus nemorosus	Ranunculaceae	6.0	1748	6	5	x	100	5.6	1.34	83.5
Rumex alpinus	Polygonaceae	5.2	1748	8	6	9	100	9.9	1.123	27.0
Scabiosa lucida	Dipsacaceae	4.2	1631	9	4	3	100	10.9	1.941	33.4
Sesleria albicans	Poaceae	3.6	1503	7	4	3	100	5.8	1.03	38.2
Silene acaulis	Caryophyllaceae	2.9	1503	9	4	1	100	13.8	0.597	28.4
Silene dioica	Caryophyllaceae	8.2	800	x	6	8	100	3.4	0.661	38.0
Silene nutans	Caryophyllaceae	6.2	1748	7	3	3	100	6.8	0.258	30.2
Silene vulgaris	Caryophyllaceae	4.7	1631	8	-	4	96	9.8	1.043	31.3
Soldanella alpina	Primulaceae	3.7	1631	7	7	x	100	9.7	0.265	-
Solidago virgaurea	Asteraceae	4.9	1631	5	5	4	100	8.9	0.424	35.5
Tofieldia calyculata	Melanthiaceae	4.2	1631	8	8	2	100	9.4	0.031	14.5
Trisetum flavescens	Poaceae	7.2	1612	7		5	100	5.0	0.18	22.8
Veronica fruticans	Scrophulariaceae	4.4	1631	8	4	2	96	13.4	0.131	-
Willemetia stipitata	Asteraceae	6.0	1748	9	4	4	96	10.0	0.706	28.4

Phylogenetic signal in the trait variation

The low and not significant *K*-values (*K*=0.13, p=0.42; Table 6) calculated for T_{min} indicate that the trait is not phylogenetically constrained. However, a significant estimate of Moran's I (I = 0.09, p = 0.032) at the family group level suggested an association between the trait and phylogeny within families with more than 4 species in our data set (*Asteraceae, Poaceae, Caryophyllaceae* and arbitrary group "other families"). A phylogenetic signal was not detected in the seed coat thickness and weight data irrespective of the method implemented, indicating a lack of association of the traits with phylogeny (Table 6).

Table 6. Summary of phylogenetical signal using different comparative methods for the initial temperature of germination (T_{min}), seed weight and seed coat thickness. *K* values range from no phylogenetic signal with $K = 0$ to high phylogenetic signal with $K >= 1$. Moran's *I* provides an estimate of the autocorrelation found within the dataset at four taxonomic and one arbitrary levels: class, subclass, order, family and arbitrary level "family group". Significance levels: * $0.01 < p < 0.05$.

Trait	**K-statistic**	**Moran's I**				
		Class	**Subclass**	**Order**	**Family**	**Family group**
Tmin	0.13	-0.01	0.03	-0.43	0.13	0.09*
Coat	0.17	-0.04	-0.04	0.002	0.02	-0.04
Weight	0.20	-0.03	-0.08	0.04	0.01	-0.06

Relationship between T_{min} and MAT

The minimal adequate model revealed that T_{min} values are strongly negatively correlated to MAT (Figure 9; $r^2 = 0.57$, $F = 7.69$, $df = 48$, $p < 0.001$). The significant interaction between MAT and family groups indicate that the intercepts and the slopes of the regression lines between MAT and T_{min} vary among different family groups (Table 7). As parameter estimations for slopes (*Caryophyllaceae*) and intercepts (*Caryophyllaceae* and *Poaceae*) were significant, three regression models were set up: one for *Caryophyllaceae*, one for *Poaceae* and one for both *Asteraceae* and other families (Figure 10).

Effects of precipitation, habitat preferences (expressed as Ellenberg indicator values) and seed traits (weight and seed coat thickness) on T_{min} – MAT relation were not detected

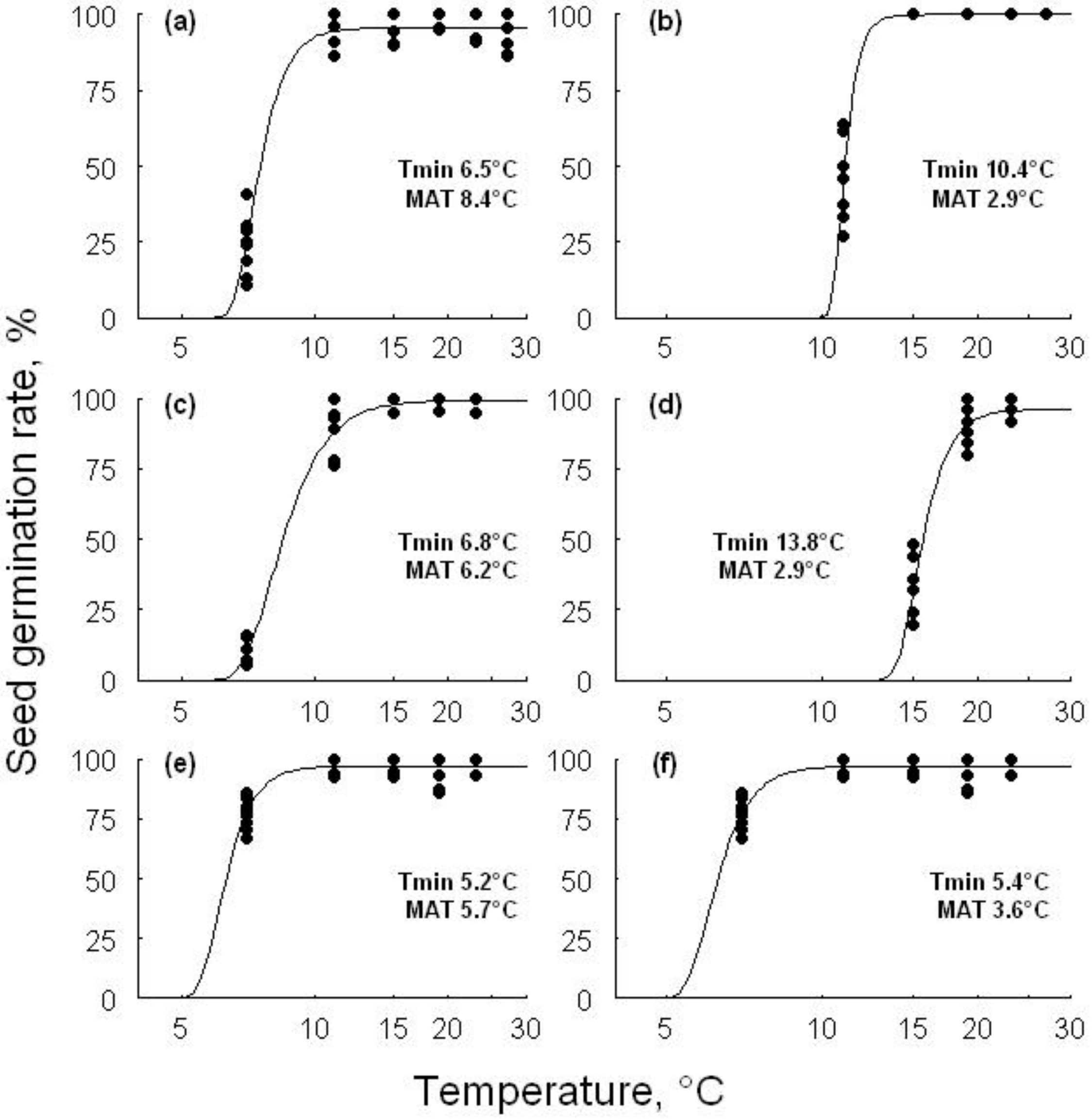


Figure 8. Germination along temperature gradient. Displayed are final germination rates together with fitted curves (Weibull function) of six species: a – *Achillea millefolium*, b – *Dryas octopetala*, c – *Silene nutans*, d – *Silene acaulis*, e – *Deschampsia cespitosa*, f – *Poa alpina*. T_{min} is initial temperature of germination (temperature under which first 5 % of all seeds germinated), MAT – mean annual temperature of collection sites. For details on seed germination experiments and curve fitting see Materials and Methods section.

Discussion

Seed germination is an irreversible process: once germination is triggered, a seedling must establish or die. Therefore, in order to increase survival chances of the seedling, time of seed germination should match environmental conditions favorable for further plant establishment (Grubb 1977; Fenner & Thompson 2005; Poschlod *et al.* 2013). Negative temperature stress is one of the crucial factor for seedling establishment, due to their high sensitivity to frost injuries (Sakai 1987; Neuner, Bannister & Larcher 1997). Consequently, seed germination should be triggered by temperatures which indicate the onset of conditions favorable for seedlings emergence. Our study reveals that T_{min} is strongly negatively correlated to habitat temperature; among the studied species T_{min} clearly increased with decreasing MAT ($r^2 = 0.57$, $p<0.001$; Figure 9). These results, therefore, confirms our original hypothesis and the observations of Thompson (1970a), suggesting that initial temperature of seed germination is a good predictor of species occurrence along temperature gradient. Habitats with high MATs are characterized by fast onset of favorable for seedling establishment temperature conditions already in early spring, namely relatively low probability of late-frost events and fast temperature rise (Table 8; Figure 11). Therefore, seed germination in these habitats may already begin shortly after snow melt that is reflected in relatively low T_{min} values of corresponding species (Table 5). With decreasing MAT, when moving along altitudinal or latitudinal gradients, frequency of late frosts as well as duration of periods with temperatures negative for plant growth are increasing (Table 8; Figure 11). Therefore, seed germination already after snow-melt involves a high risk of seedling loss (Billings & Mooney 1968; Sakai 1987; Shimono & Kudo 2005). In order to reduce this risk, germination is triggered by relatively high ambient temperatures in late spring or early summer, which indicate the onset of favorable conditions for further seedling recruitment (Fenner & Thompson 2005).

However, the pattern detected here might not be universal, because the seed samples used in this study originate from a temperate climate zone. In other climates (e.g. Mediterranean), temperature stress (here cold or frost, both the main hazard for seedling establishment in temperate regions) may be replaced by water stress which is drought. In regions with Mediterranean climate seeds tend to germinate under lower temperatures of wet winters, because hot dry summer conditions limit water availability and, thus, further seedling recruitment (Fenner & Thompson 2005) (Cochrane, Daws & Hay 2011). This may explain, why the amount of precipitation per collection site did not have any significant effect on T_{min} variation in our data set. In the study region water does not play a limiting role for our species; all collection sites receive sufficient amounts of precipitation (800-1500 mm per year, Table 5), which are distributed without strong irregularities over the year (Marke *et al.* 2013).

Therefore, the character and power of the T_{min} – MAT relation detected in our study may be true for temperate regions but should be tested under other climatic conditions.

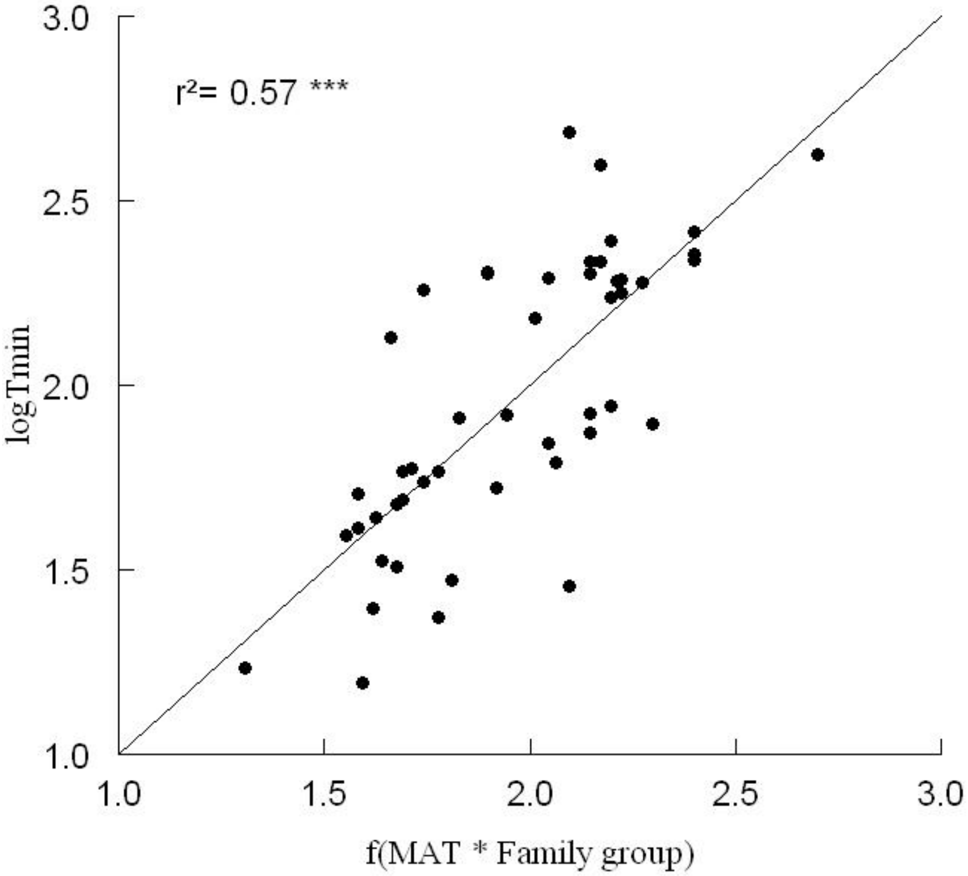


Figure 9. Graphical representation of the results of the linear model investigating the effect of MAT on T_{min} (r^2= 0.57, F=7.69, df=48, p<0.001). MAT is mean annual temperature. Family group is an arbitrary group including plant families with more than 4 species in our data set (*Asteraceae*, *Poaceae*, *Caryophyllaceae* and “other families”).

Phylogenetic signal in the trait variation

Since seed germination patterns are often phylogenetically constrained (Baskin & Baskin 1988; Baskin & Baskin 1998; Milberg, Andersson & Thompson

2000), making any conclusions on a trait-environment relation suspect, we also analyzed the T_{min} variation along the gradient of MAT after taking phylogeny into account. Applied tests revealed moderate differences (Moran's *I* = 0.09, p= 0.032) in the T_{min} – MAT relation among families with more than 4 species (*Asteraceae*, *Caryophyllaceae* and *Poaceae*) in the data set. The inspection of the regression lines drawn for every family group (Figure 10) indicated that species from *Asteraceae* family show the same character and strength of T_{min} – MAT relation as species from the arbitrary group "other families", while those for *Caryophyllaceae* and *Poaceae* were found to be different.

Table 7. Estimated parameters for slopes (m) and intercepts (B) of the different family groups (output of the linear model) for the $T_{min} \sim$ MAT relation. "Asteraceae" is the null model. Derived slopes (m') and intercepts (B') for the regression lines of the different family groups are given.

	m	T	p-value	B	T	p-value	m'	B'
Asteraceae	-0.101	-2.85	0.006	2.51	11.54	<0.001	-0.101	2.51
Caryophyllaceae	-0.162	-2.05	0.047	0.96	2.05	0.047	-0.263	3.47
Other	-0.051	-0.92	0.36	0.33	1.09	0.28	-0.101	2.51
Poaceae	0.07	1.12	0.27	-0.71	-1.90	0.065	-0.101	1.80

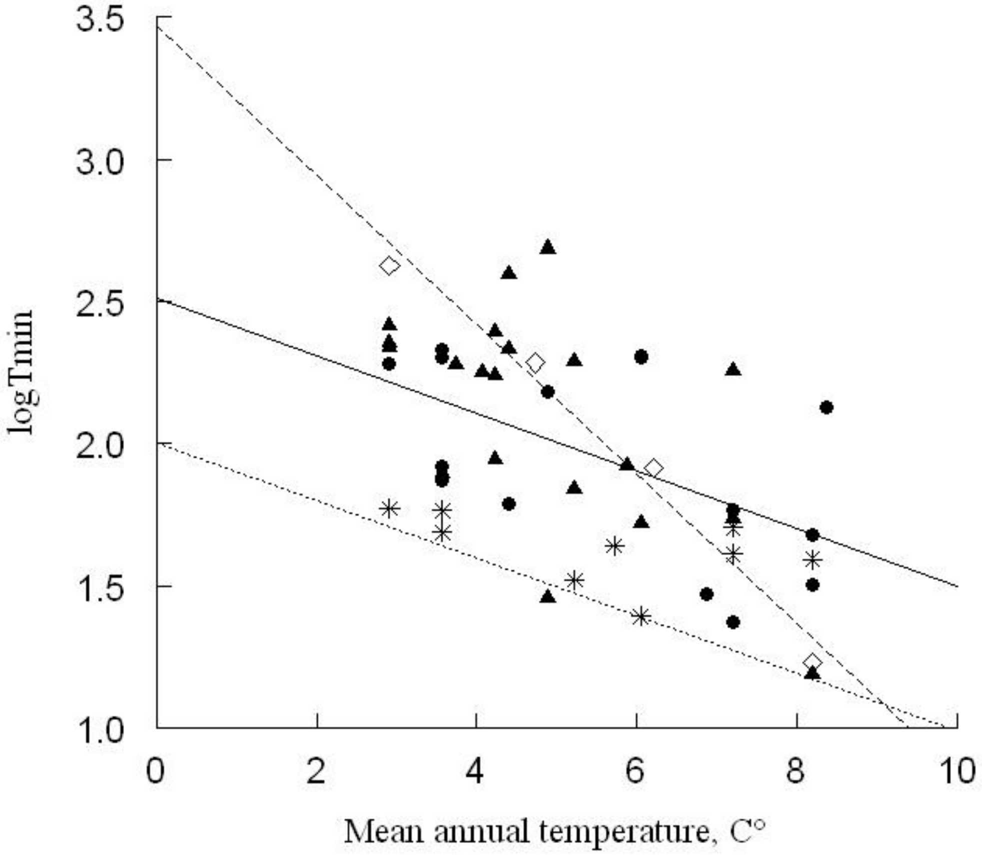


Figure 10. Regressions of log-transformed initial temperature of germination to habitat MAT with regard to the three different family groups: • Asteraceae, ◊ Caryophyllaceae, * Poaceae, ▲others. For each family group, the regression lines are given (solid line: Asteraceae and others, dotted line: Caryophyllaceae, dashed line: Poaceae).

Similar slope of the *Poaceae* regression line indicates the difference in T_{min} values between lowland and alpine grasses, however, relatively small intercept shows that grasses start to germinate generally under lower temperatures than other species. This behavior can be related to their competitive strategy; early germination has a strong competitive advantage (Grime 2002). Although T_{min} of

Caryophyllaceae is in the same manner related to MAT of habitats, a steeper slope and relatively big intercept indicate that the power of this relation is stronger in the family that is in line with the previous finding (Thompson 1970c). Similar directions of the regression slopes (in all cases T_{min} values increase with decreasing MAT) and different intercepts, therefore, suggests that the T_{min} - MAT relation has an universal character among all studied plant families with family-specific ranges of the T_{min} values.

Effect of other than temperature environmental factors on the T_{min} - MAT relation

Due to the fact that local climatic conditions may significantly modify seed germination response to ambient temperature (Grubb 1977; Schütz & Rave 1999), we tested for variation in T_{min} – MAT relation among species with various habitat preferences expressed by Ellenberg indicator values (EIV). Results of the final adequate model (Figure 9) indicate that there were no differences in temperature response of germination within species with various habitat preferences in light (EIV L), soil moisture (F) and soil fertility (N). These results may be explained in the first place by the stronger influence of ambient temperatures on seed germination in comparison to other environmental triggers. However, all tested species originate from the same vegetation type, namely open nutrient poor calcareous grasslands, where there are no distinct differences in irradiation levels, soil fertility, soil moisture and soil reaction as well. Therefore, the T_{min}-MAT relation might rather differ among species originating from different habitat types (e.g. forest, wetlands, steppe, etc.).

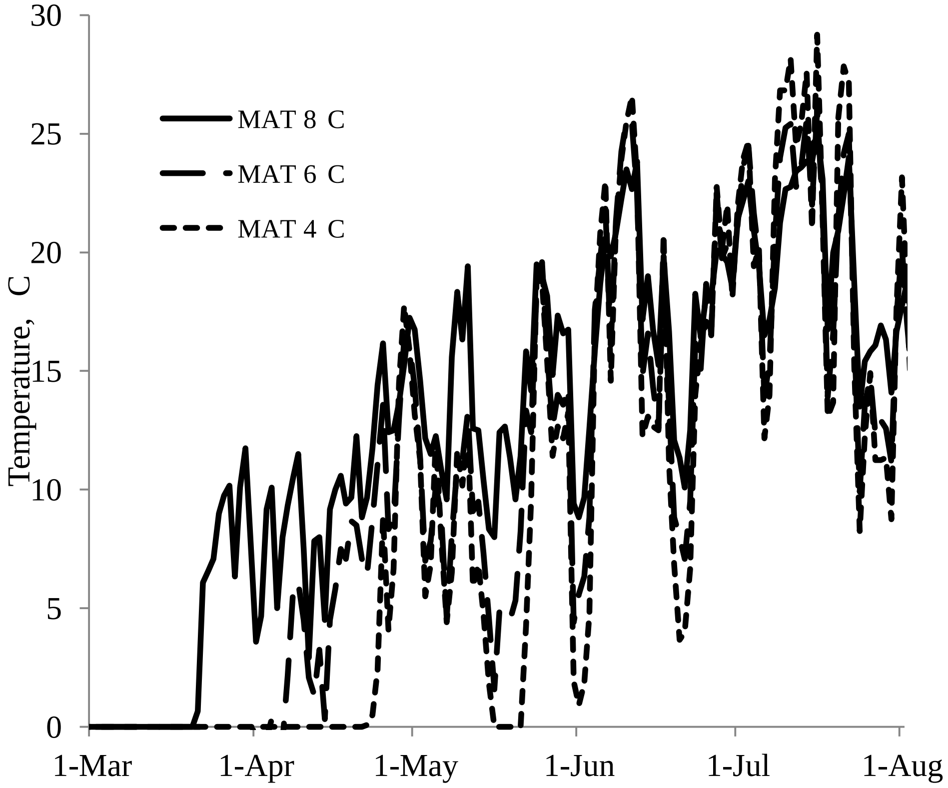


Figure 11. Soil surface temperatures of three seed collection sites with different MATs (Courtesy of Annette Leingärtner and Bernhardt Hoiss). The measurements were carried out in the National Park Berchtesgaden in 2010 with the help of temperature loggers (Thermochron iButtons DS1921G#F5, Maxim Integrated Products, Inc., Sunnyvale, CA, USA).

Coevolution of seed traits

Despite the fact that seed morphological and germination traits may be coevolved (Jurado & Westoby 1992; Bond, Honig & Maze 1999; Schütz & Rave 1999; Milberg, Andersson & Thompson 2000), we did not find any differences in the T_{min}– MAT response among species differing in seed mass and seed coat thickness. Our data suggest that seed morphological traits and T_{min} are not coevolved, because they are probably responsible for different functions: seed coat for seed persistence in soil (Gardarin *et al.* 2010) and partly for seed dormancy (physical dormancy (Baskin & Baskin 1998; Baskin & Baskin 2004)), T_{min} for germination cueing under favorable temperature conditions (Fenner & Thompson 2005) and seed weight for seed dispersal and seedling establishment (Gross 1984; Poschlod *et al.* 2013).

Conclusions

The distribution and abundance of plant species are intimately related to their reproductive success which in turn is extremely sensitive to environmental conditions (Grubb 1977; Fenner & Thompson 2005; Bykova *et al.* 2012; Poschlod *et al.* 2013). The strong relation between T_{min} and habitat temperature conditions, proven in the present study, clearly support the important role of seed ecology in mechanistic understanding of plant ecology and distribution. This implies that the T_{min}-MAT relation can particularly provide insights into species distribution patterns, vegetation dynamics and community assembly rules along altitudinal and latitudinal gradients. Furthermore, including the T_{min} in species distribution models (Bykova *et al.* 2012) may help to improve the accuracy and specificity of predictions of vegetation shifts under global change scenarios.

Table 8. Characteristics of sites with different MATs. The number above is mean month temperature, the number below number of days with negative temperatures (frost events). MAT were obtained from the weather stations located in the National Park Berchtesgaden and presented as mean values for the last 10 years of observation.

Mean annual temperature, C°	**January**	**February**	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
8	-1.3 19	0.3 12	3.7 5	7.6 1	13.1 0	15.5 0	16.9 0	17.1 0	12.6 0	8.1 1	2.6 8	-0.9 19
7	-2.7 25	-1.3 17	2.2 7	6.6 1	12.1 0	14.8 0	15.7 0	15.7 0	10.7 0	7.5 1	2.7 8	-1.3 20
6	-2.1 19	-0.5 14	1.1 13	5.3 5	10.6 0	12.7 0	14.5 0	14.2 0	9.9 0	6.7 2	1.4 11	-0.9 17
5	-2.4 21	-1.7 17	0.5 14	3.6 7	8.9 1	11.3 0	12.6 0	13.0 0	8.7 0	5.9 3	0.6 14	-1.9 19
4	-2.1 20	-2.2 18	-0.3 15	2.5 9	7.2 1	9.9 0	11.3 0	12.0 0	7.9 1	5.3 3	0.8 13	-1.5 19
3	-4.0 25	-3.7 21	-1.5 18	1.1 12	6.1 3	8.9 1	9.9 0	11.0 0	7.0 2	5.0 5	-0.5 15	-2.6 20

Chapter 5

Altitude matters – contrasting effects of climate change on the vegetation development at different elevations in the Bavarian Alps

Abstract

Despite broad evidence that recent climate change considerably affected alpine-nival vegetation, there are only a few studies revealing climate-induced vegetation changes in all vegetation belts above tree-line. Here, we use historical vegetation surveys from the Bavarian Alps, in order to examine biodiversity, structural and functional trait composition changes in subalpine, lower and upper alpine vegetation belts in last 50 years. The results of our study indicate that the direction and magnitude of these changes differed considerably among the studied communities and were dependent on initial species composition, vegetation structure and geographical context of individual vegetation belts. Although the species richness did not change significantly over last 50 years in the dense subalpine *Seslerio-Caricetum sempervirentis* communities, the immigration of species from lower altitudes and increased environmental favorability for already present thermophilous species led to increase of plant cover (+23%). The most pronounced changes were found in sparse low alpine

Caricetum firmae communities. Due to gap availability and low competitive ability of alpine species, significant increase in species richness and plant cover (+6.4 species and +18%, respectively) were detected in this vegetation belt. The RLQ analysis revealed that in both communities species with traits that are advantageous under warmer conditions, such as higher specific leaf area, high stature and heavier seeds, significantly increased their frequencies and abundances. Floristic and vegetation changes of upper alpine communities were contrasting to those found in the study region as well as in the close mountain ridges. Despite temperature increase and available for colonization gaps in the communities, species richness (–4.7 species) and plant cover (–25%) significantly decreased. We argue that increased instability of high alpine habitat, due to upward retreat of the permafrost zone (as a consequence of recent warming), could hamper the colonization of high altitudes by montane and subalpine species, since they demand developed soils with high water-retention capacity. Our results suggest that the most often reported patterns of vegetation change found in the alpine-nival ecotone may be very different at lower altitudes, due to the differences in species pools, vegetation structure and environmental characteristics of the corresponding vegetation belts.

Introduction

There is ample evidence that ongoing climate change continuously affects alpine vegetation with its different components (Grabherr, Gottfried & Pauli 1994; Theurillat & Guisan 2001; Walther *et al.* 2002; Gottfried *et al.* 2012). Shifts in plant life-history (McKone, Kelly & Lee 1998), disruptions of biological relationships (Klanderud 2005) and general upward shifts of species' distributional ranges (Walther, Beißner & Burga 2005; Jurasinski & Kreyling 2007; Pauli *et al.* 2012) belong to the responses of alpine vegetation to recent global warming which are reported most. These changes, due to highly specific ecological conditions (Körner 1999; Nagy & Grabherr 2009) and low competitive ability of cryophilous species, could lead to a drastic decrease of the distribution area or the extinction of alpine species (Pauli, Gottfried & Grabherr 2003) and even complete habitat loose of alpine vegetation (Thuiller *et al.* 2005; Gottfried *et al.* 2012). That is why this problem is of high importance for nature conservation and management decisions around the world, since the recent climatic changes have already affected a broad range of mountain regions across all continents, except of the Antarctic (Walther *et al.* 2002; Danby *et al.* 2011; Jump, Huang & Chou 2012; Pauli *et al.* 2012; Venn, Pickering & Green 2012).

The European Alps are considered to be highly vulnerable to climate change (Haeberli & Beniston 1998; Theurillat & Guisan 2001; Walther *et al.* 2002). Numerous studies provided evidence for consistent increase of plant species richness (Grabherr, Gottfried & Pauli 1994; Erschbamer *et al.* 2009; Pauli *et al.* 2012), thermophilization of the flora (Gottfried *et al.* 2012), increase of plant cover (Pauli *et al.* 2007) and vegetation homogenization (Jurasinski & Kreyling 2007). Changes in species richness, community structure and composition as well as functional characteristics of high mountain plant communities are predicted to continue in the future (Thuiller *et al.* 2005; Engler *et al.* 2011; Dullinger *et al.* 2012). However, an overall picture of vegetation changes above the tree line in response to recent climate change is still unavailable. Many studies reported biodiversity changes in high alpine and nival vegetation belts (e.g. (Grabherr, Gottfried & Pauli 1994; Pauli *et al.* 2007; Wipf *et al.* 2013)), while only few studies focus on the vegetation of lower altitudes, such as low alpine and subalpine belts. Although high alpine and nival vegetation are considered to be a sensitive ecological indicator for climate change effects (Pauli, Gottfried & Grabherr 2003), conclusions drawn from these studies cannot be fully applied to vegetation of low alpine and subalpine belts. Differences in environmental characteristics (e.g. deep, well-drained soils in subalpine belt vs. rocky screes of high altitudes), structure of plant communities (decreasing density of plant cover with increasing elevation) and size of neighbor species pools (higher at low elevations) may significantly modify direction and magnitude of responses of vegetation originating from different altitudes to recent climatic change (Cannone, Sgorbati & Guglielmin 2007;

Erschbamer *et al.* 2009; Vittoz *et al.* 2009; Pauli *et al.* 2012). For example, effects of climatic change were found to be less pronounced in subalpine grasslands than have been previously observed in alpine environments, due to low levels of invasion susceptibility of communities with no available gaps for establishment of newly arrived species (Vittoz *et al.* 2009).

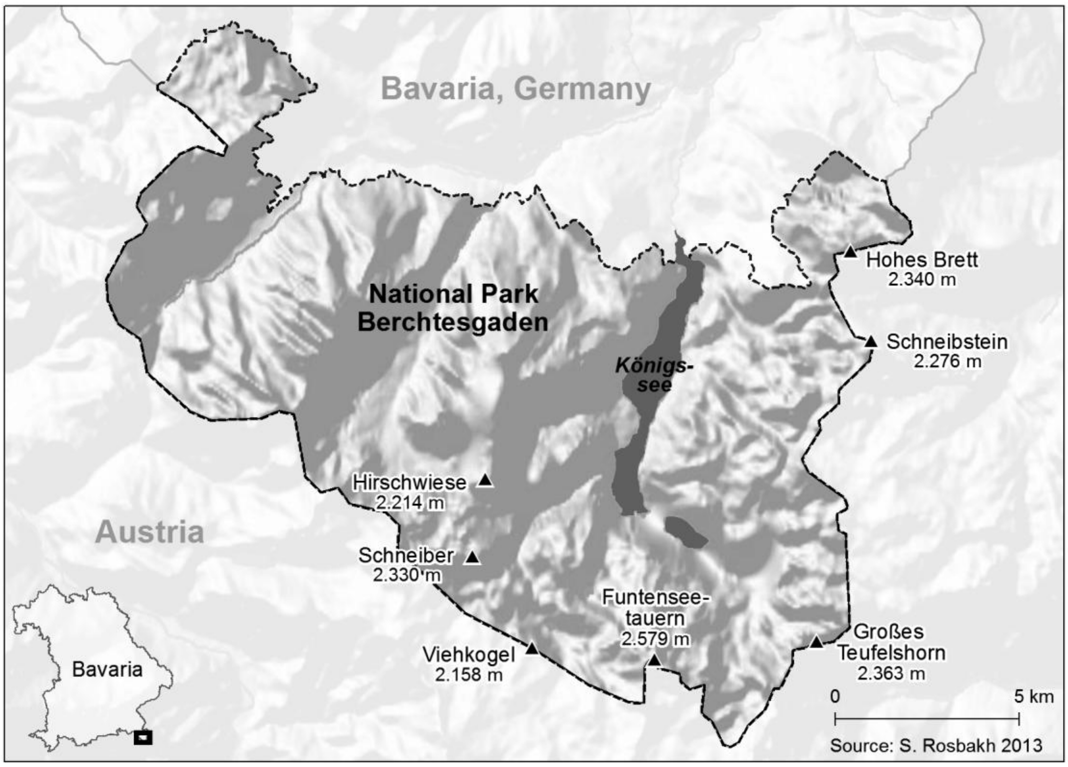
Here, we re-visited historical vegetation surveys (1961) from the Bavarian Alps, in order to analyse biodiversity and structural changes of subalpine and alpine species-rich calcareous grasslands during the second half of the 20th century. According to already published evidences for floristic and vegetation changes in nearby mountain areas (Kudernatsch *et al.* 2005; Pauli *et al.* 2007; Erschbamer *et al.* 2009; Pauli *et al.* 2012), we hypothesized that the changes driven by climate warming were less pronounced at lower altitudes (subalpine grasslands) as compared to alpine communities, owing to differences in environmental conditions, community structure and sizes of neighbor species pools. In order to lighten the mechanisms and processes behind these changes, we also used plant functional traits. We expected that species with traits that are advantageous under warmer conditions increased their presence and/or frequency in all studied communities in the last 50 years as a result of thermophilization high mountain vegetation, a processed caused by decline of cryophilous and decrease of thermophilous species (Gottfried *et al.* 2012).

Materials and methods

Study sites and vegetation data

The study was carried out in the Berchtesgaden National Park (Bavaria, Germany), which is situated in the Bavarian Alps (northern part of the Calcareous Alps) encompassing an area of approximately 200 km². The relief of the National Park can be characterised as typically alpine, with steep mountain peaks composed of Triassic lime and dolomite rocks (Marke *et al*. 2013). The climate is a typical mountain climate, showing a large decrease in mean annual air temperatures from +7° to –2°C as elevation increases from 603 to 2713 m above sea level (a. s. l.). Mean annual precipitation ranges from approximately 1500 to 2600 mm per year (Marke *et al*. 2013).

The vegetation of the National Park was mapped between 1961 and 1963 by Wolfgang Lippert (Lippert 1966), based on a set of about 1000 vegetation surveys following the method of Braun-Blanquet (Braun-Blanquet 1964). Although the positions of the historical surveys were not permanently marked in the field, their locations were precisely described (e.g. landmarks, altitude, exposition, inclination) that allowed a reliable re-identification in the field (semi-permanent plots; Kudernatsch *et al.* 2005). We extracted 35 surveys from the original data set of seven summits (Figure 12), belonging to the vegetation types *Seslerio-Caricetum sempervirentis* (14 surveys; subalpine vegetation belt) and *Caricetum firmae* (21 surveys; alpine vegetation belt). Because of high heterogeneity of ecological conditions in the alpine belt (temperature, soil nutrition, disturbance, etc.; (Oberdorfer 1978; Kudernatsch *et al.* 2008)), surveys of the *Caricetum firmae* were divided into two groups: those of lower (CFL) and upper (CFU) altitudes (threshold: 2300 m a.s.l.). The characteristics of the studied communities are given in Table 9.



Source: S. Rosbakh 2013

Figure 12. Location of the study sites in the Berchtesgaden National Park (Bavarian Alps, Germany). Detailed characteristics of the vegetation surveys are given in Appendix 8.

In August to September 2012, we re-surveyed the historical relevés, retaining original location (altitude, exposition, surface morphology and plot size) and following the same method as Lippert did (Lippert 1966). Taxonomy was unified using the nomenclature as in Schönfelder and Bresinsky (1990).

Trait data

Based on the LHS-scheme proposed by Westoby (1998), we selected key plant functional traits related to growth, persistence and reproduction of plants, namely, specific leaf area (SLA), canopy height and seed mass. SLA (leaf area divided by its dry weight) is related to the potential growth rate and ecological strategy of plants (Westoby 1998; Reich *et al.* 1999; Poorter *et al.* 2009). Canopy height is a key trait that is related to light access and, therefore, to the competitive ability and persistence of plants (Westoby, 1998). Seed mass is a good indicator of species' ability to disperse, survive and establish under various hazards (Westoby 1998). The variation of these functional traits determines the location of species along resources gradients and adaptation ability to low temperature stress (Westoby 1998; Grime 2002; Venn *et al.* 2011; De Frenne *et al.* 2013). They are especially suitable to estimate vegetation changes in alpine habitats, because their temperature-relevance have been already proven in previous studies (e.g. (Wright *et al.* 2004; Pellissier *et al.* 2010; De Frenne *et al.* 2013))

Based on the historical and recent vegetation surveys, a complete list of species (in total 98) occurring within the target communities was compiled and trait measurements of all species were subsequently carried out in the field according to generally accepted methods (Pérez-Harguindeguy *et al.* 2013). Missing values for SLA and seed mass (8 species each) were obtained from the LEDA trait-base (Kleyer *et al.* 2008).

Table 9. Characteristics of the studied communities (Oberdorfer 1978; Kudernatsch *et al.* 2005; Ellenberg & Leuschner 2010). Information on altitudinal distribution of different communities and average species number within the community are given for the study area.

Characteristic	Community		
	Seslerio-Caricetum sempervirentis	*Caricetum firmae* lower altitudes	*Caricetum firmae* upper altitudes
Altitude, m a.s.l.	1600-2200	2100-2300	2300-2600
Vegetation belt	Subalpine	Low alpine	High alpine
Soil	Deep, well-drained soils	Shallow initial soils, rocky screes	
Permafrost	no	no	yes
Snow pack	Thick	Thin at southern slopes, thick at northern slopes	
Plant cover	Dense	Sparse	Scattered
Average species number per community	30	21	15
Typical species	*Sesleria albicans*	*Carex firma*	*Carex firma*
	Carex sempervirens	*Silene acaulis*	*Dryas octopetala*
	Poa alpina	*Bistorta vivipara*	*Arctostaphylos alpinus*
	Anthyllis alpestris	*Festuca quadriflora*	*Salix retusa*
	Galium pumilum	*Ranunclus alpestris*	*Primula minima*

Data analysis

Biodiversity changes over time

In order to estimate temporal changes in biodiversity, we calculated for each pair of historical and recent relevés three different measures: 1) number of not found and newly found species; 2) species richness (number of occurring species) and 3) species evenness following Smith and Wilson (1996). The index was used, because it is independent on species richness and sensitive to both rare and common species (Smith & Wilson 1996). Additionally, differences in species composition between old and new vegetation surveys were described by the Bray-Curtis similarity index (Bray & Curtis 1957). For each plant community, differences in species richness and species evenness between the historical and recent surveys were tested using paired t-tests. For the number of not found and newly found species, differences between communities were tested using an ANOVA.

Changes in vegetation structure over time

To identify changes in vegetation structure over time, RLQ-analyses were performed. This kind of ordination analyses links vegetation data, species and environmental information (Dolédec *et al.* 1996). For the analysis, data on habitat properties, community composition and traits were organized in three tables. The habitat table (R) contained 35 sites x 5 environmental factors (year of vegetation survey, altitude, slope, exposition divided into a north/south and west/east component). The community data table (L) contained 35 sites x 98 species with the estimated cover values (ranging from 0 to 1). The trait table (Q) contained 98 species x 3 traits (SLA, canopy height, seed mass).

As the first step of the analysis, a correspondence analysis (CA) was performed on the L-table after arcsine-square-root transformation of the proportional cover values. Second, Hill-Smith mixed ordinations (Hill & Smith 1976) were carried out on the R-table using the row scores of the CA on L-table as canonical factor and on the Q-table using the column scores of the CA on the L-table as canonical factor. Subsequently, the RLQ calculates two separate co-inertia analyses, one between the constrained ordination on the Q-table and the CA on L and the second between the constrained ordination on the R-table and the CA on L. In an iterative process RLQ-axes were determined, which display maximum covariance between the Q- and the R-axes (Dolédec *et al.* 1996).

Changes in functional trait composition over time

To define functional groups responding to the potential changes in climatic conditions, we used the species scores of the RLQ-analysis. A grouping was done by k-means clustering, with subsequent bootstrapping (Jaccard-distance) to choose an appropriate cluster number (Hennig 2009). Subsequently, we characterized each cluster by its trait mean attributes and standard error. Differences among the clusters were tested using randomization tests: for each trait 1000 randomizations were performed and the difference to the observed mean was calculated; if random samples showed in less than 0.05 of all permutations a mean lower than observed, a significant difference was assumed. Additionally, the loadings of the environmental factors of the RLQ-axes were used to estimate the relative meaning of each single factor for describing the vegetation development represented by the RLQ-ordination (Bernhardt-Römermann *et al.* 2008). All calculations were made using the libraries ade4 (Dray & Dufour 2007), vegan (Oksanen *et al.* 2013) and ftc (Hennig 2009) in R version 2.14.2 (R Development Core Team 2012).

Results

Biodiversity changes in time

Changes in numbers of not found and newly found species as well as species richness were community specific. Due to similar number of not found and newly found species (13.4 and 14.3, respectively; Figure 13a), species richness of surveys belonging to SES communities did not change significantly over last 50 years (Figure 13b). In contrast, in CFL communities a highly significant increase of species richness (+6.4 species; Figure 13b) was detected. This difference refers to the higher number of newly found species in comparison to that of not found ones (12.5 and 6.1, respectively; Figure 13a). An opposite trend was observed for the CFU communities: species richness significantly decreased during the last 50 years (6.4 species; Figure 13b). Again, an explanation is provided by the number of not found species and newly appearing ones (10.7 vs. 6; Figure 13a).

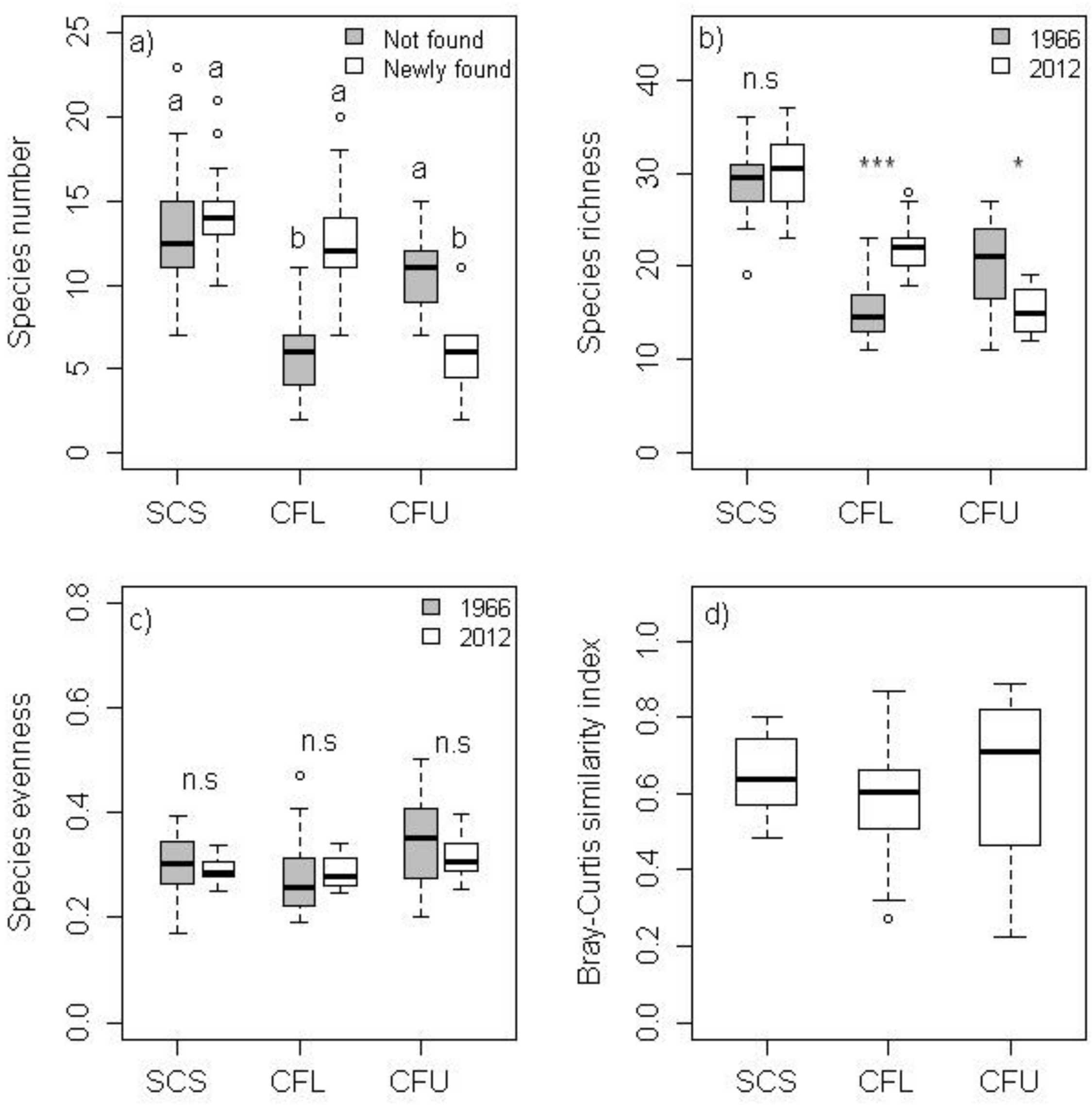


Figure 13. Changes in species diversity during the last 50 years separated by the communities *Seslerio-Caricetum sempervirentis* (SCS), lower and upper plots of the *Caricetum firmae* (CFL, CFU): **a**) number of not found and newly found species, **b**) species richness, **c**) species evenness and **d**) changes in vegetation composition. Letters indicate statistical differences among studied communities ($p<0.05$, one-way ANOVA). Asterisks indicate significant differences between historical and recent data (paired t-test: *** - $p<0.001$, * - $p<0.05$,).

When comparing changes in species evenness (Figure 13c), no significant difference was found in any community (SES =0.01, CFL=0.004, CFU=-0.03).

The Bray-Curtis index calculated for vegetation changes during the last 50 years indicates that all target communities changed their vegetation composition (Figure 13d; SES=0.65, CFL=0.58, CFU=0.63).

Changes in vegetation structure in time

All communities studied changed over time; a clear separation between historical and recent relevés is visible in the RLQ-ordination diagrams (Figure 14a, 14c, 14e). Next to age, sites were also separated through their different ecological conditions: for SES exposition (north, east), and for CFL and CFU exposition (north, east), slope, and altitude were significantly related to differences in the community structure.

Vegetation cover increased during the last 50 years in SES and CFL communities from 78% to 100% and from 67% to 85%, respectively (Table 10; correlation with axis 1: r=0.82, axis 2: r=0.56 and with axis 1: r=0.86, respectively). An opposite pattern was detected for the CFU communities; vegetation cover has decreased over time from 75 to 65% (Table 10; correlation with axis 1: r=0.85).

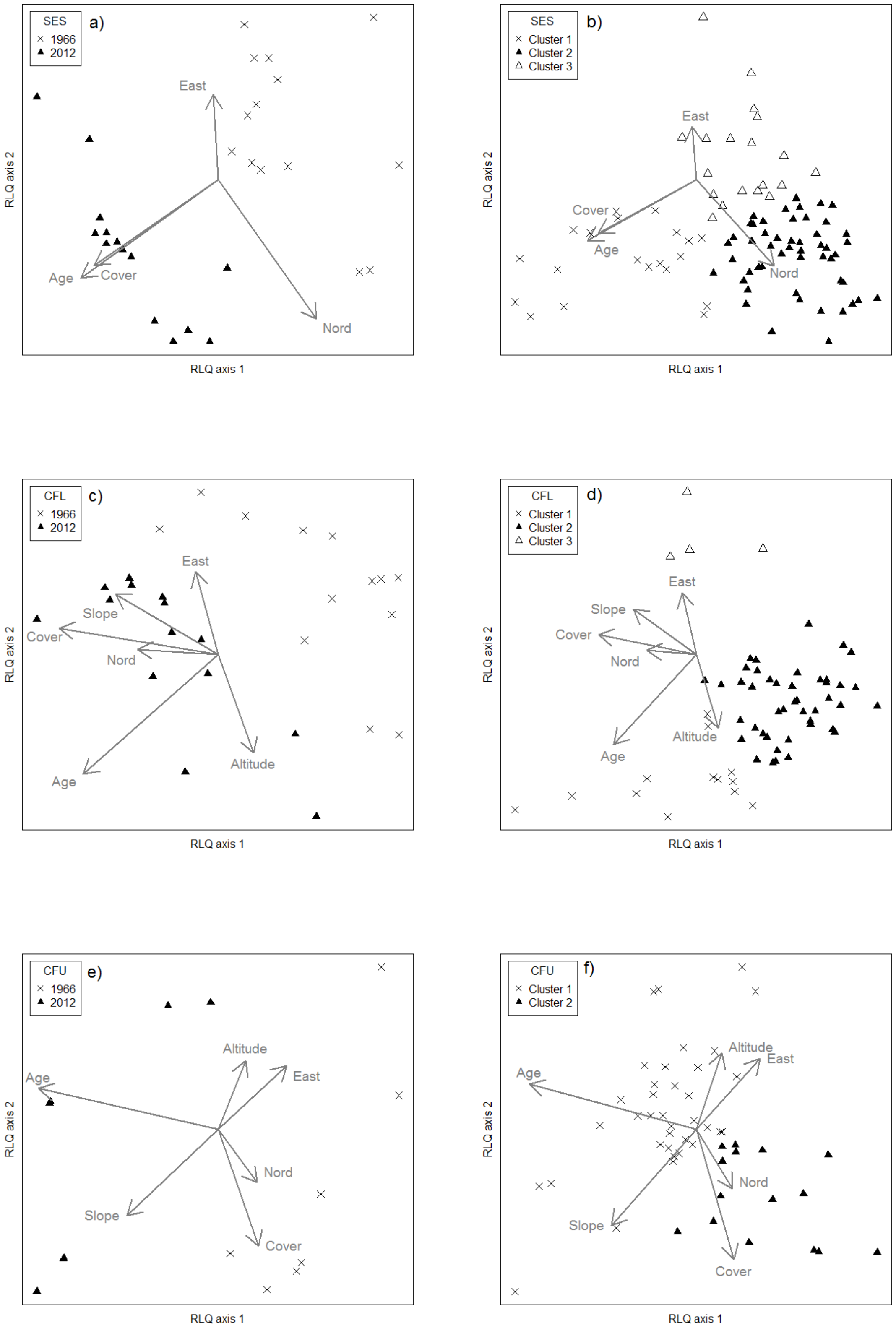


Figure 14. Results of the RLQ-analyses for the *Seslerio-Caricetum sempervirentis* communities (SES; 14a, 14b), lower and upper stands of the *Caricetum firmae* (CFL, CFU, 14c, 14d and 14e, 3f, respectively. Figure 14a, 14c, 14e show the vegetation differentiations between historical and recent relevés, while Figure 14b, 14d, 14f show the species scores

divided into species groups (taken from the cluster analyses). Significant correlations between the axes and environmental factors are plotted (compare Table 10).

14a, 14b: Results for the SCS communities. The first two RLQ-axes represent 96.5% of the variance explained by the CA on the L-table. 1. axis: Eigenvalue: 0.051; 2. axis: Eigenvalue: 0.013. L-table: 91 species vs. 28 plots; R-table: 28 plots vs. 8 environmental factors; Q-table: 91 species vs. 3 traits.

14c, 14d: Results for the CFL communities. The first two RLQ-axes represent 98.6% of the variance explained by the CA on the L-table. 1. axis: Eigenvalue: 0.18; 2. axis: Eigenvalue: 0.01. L-table: 61 species vs. 28 plots; R-table: 28 plots vs. 8 environmental factors; Q-table: 61 species vs. 3 traits.

14e, 14f: Results for the CFU communities. The first two RLQ-axes represent 96.4% of the variance explained by the CA on the L-table. 1. axis: Eigenvalue: 0.05; 2. axis: Eigenvalue: 0.004. L-table: 51 species vs. 14 plots; R-table: 14 plots vs. 8 environmental factors; Q-table: 51 species vs. 3 traits.

Changes in functional trait composition in time

The cluster analysis deduced for each vegetation type stable groupings of species responding to recent climate change (Figure 14b, d, f; vector “age”). Additionally to the temporal changes, the RLQ analysis also revealed in SES and CFL communities groupings of species related to environmental heterogeneity within corresponding vegetation belts. The characteristics species of the groups and their mean trait attributes are shown in Table 11.

In the SES communities, species representing cluster 1 (occurring on sites of the new observation with higher vegetation cover, Figure 14b) are significantly taller (0.29 vs. 0.09 and 0.07 cm) and produce significantly heavier seeds (2.22 vs. 0.86 and 0.56 mg) in comparison to species from other two clusters (number

2 and 3, respectively). SLA values of the species from the first cluster are significantly lower in comparison to those of cluster 2 (19.7 vs. 29.1 mm^2/mg). Species from the second cluster, which tend to occur at north-western slopes, have similar plant height and seed weight as species of cluster 3; however, their SLA values were significantly higher than those of the other two clusters (29.1 mm^2/mg). Species of the third cluster tend to occupy slopes of south-eastern expositions. Functional characteristics of these species, apart from the SLA values (16.5 vs. 29.1), were statistically similar to those of the second cluster (Table 11).

Table 10. Correlation between the environmental variables and the first two axes of the RLQ axes. Bold are significant entries ($p < 0.05$)

Community	SES		CFL		CFU	
	Axis 1	Axis 2	Axis 1	Axis 2	Axis 1	Axis 2
Age	**-0.85**	**-0.56**	**-0.74**	**-0.4**	**0.88**	0.19
Cover	**-0.82**	**-0.56**	**-0.86**	0.01	-0.14	**-0.85**
Altitude	0.24	-0.32	0.29	**-0.87**	-0.26	**0.88**
North	0.2	**-0.45**	**-0.5**	0.08	0.05	**-0.81**
East	0.02	**0.58**	-0.3	**0.78**	**-0.57**	**0.86**
Slope	0.24	-0.27	**-0.72**	**0.45**	**0.68**	**-0.75**

For the CFL communities (Figure 14d), 3 groups of species were found; species of cluster 1, representing the gained species (n=12), have higher SLA values (29.4 mm^2/mg) and are taller (0.18 cm) in comparison to the other two clusters. Seed weight of the species from this cluster were not significantly different from species representing cluster 3. SLA and plant height of species from cluster 2, which occupy south-eastern slopes, did not differ from those of cluster 1; however, their seed weight was considerably higher (5.6 mg). Four species in this community, *Anthyllis vulneraria*, *Arctostaphylos alpinus*, *Biscutella laevigata* and *Hedysarum hedysaroides*, were found to prefer steep eastern slopes. Only the considerably heavier seeds (5.6 vs. 0.55) separated species from this cluster from those of the cluster 2.

The cluster analysis for the CFU communities deduced two species groups (Figure 14f). Although plant height and seed weight of gained species from cluster 1 were higher in relation to cluster 2, these differences are not significant. Moreover, they are characterized by lower SLA values (15.1 vs. 26.7) in comparison to the other cluster. Additional groupings related to environmental heterogeneity were not detected in this plant community.

Discussion

For the study period (1962-2012), we found changes in biodiversity, community structure and functional trait composition in all of our studied communities. However, initial species composition, geographical position and geographical context of individual vegetation types have resulted in dissimilar responses to climate change not being reflected in the same way in all vegetation belts.

Table 11. Characteristics of clusters obtained by k-mean clustering of species scores of RLQ analysis. Traits shown differed significantly between species of the different clusters (randomization test) and representative species per cluster are given. n – number of species per cluster, * - cluster of the gained species (see text).

	Plant community							
	SES			CFL			CFU	
Trait	Cluster 1* n= 20	Cluster 2 n= 19	Cluster 3 n= 52	Cluster 1* n= 12	Cluster 2 n= 4	Cluster 3 n= 45	Cluster 1* n= 36	Cluster 2 n= 15
Specific leaf area	19.7 ± 1 *a*	29.1 ± 1.5 *b*	16.5 ± 0.6 *a*	29.4 ± 2.1 *a*	15.1 ± 1.5 *b*	16.8 ± 0.8 *b*	15.1 ± 0.6 *b*	26.7 ± 1.7 *a*
Plant height	0.29 ± 0.03 *a*	0.09 ± 0.01 *b*	0.07 ± 0.01 *b*	0.18 ± 0.04 *a*	0.08 ± 0.02 *b*	0.07 ± 0.01 *b*	0.09 ± 0.02 *a*	0.06 ± 0.01 *a*
Seed weight	2.22 ± 0.52 *a*	0.86 ± 0.18 *b*	0.56 ± 0.08 *b*	0.36 ± 0.08 *a*	5.6 ± 1.04 *b*	0.55 ± 0.06 *a*	1.01 ± 0.28 *a*	0.37 ± 0.09 *a*
Representative species	*Aconitum tauricum*	*Cerastium fontanum*	*Achillea clavennae*	*Agrostis alpinc*	*Anthyllis vulneraria*	*Androsace chamaejasme*	*Bistorta vivipara*	*Campanula alpina*
	Carex sempervierns	*Hieracium villosum*	*Bartsia alpina*	*Campanula alpina*	*Arctostaphylos alpinus*	*Carex firma*	*Carex ornithopoides*	*Euphrasia picta*
	Luzula sylvatica	*Myosotis alpestris*	*Carex atrata*	*Gentiana aspera*	*Biscutella laevigata*	*Dryas octopetala*	*Salix retusa*	*Minuartia verna*
	Rhinanthus glacialis	*Ranunculus montanus*	*Juncus monanthos*	*Parnassia palustris*	*Hedysarum hedysaroides*	*Minuartia verna*	*Silene acaulis*	*Pritzelago alpina*
	Trifolium pratense	*Scabiosa lucida*	*Primula minima*	*Pinguicula alpina*		*Primula auricula*	*Tofieldia pusilla*	*Ranunculus alpestris*

Changes in subalpine Seslerio-Caricetum sempervirentis communites

Species distribution along altitudinal gradients is mainly controlled by ecophysiological processes of temperature tolerance (Sakai 1987; Körner 1999; Larcher 2004). The temperature rise and the lengthening of growing season observed in the Alps during the last decades have alleviated the growth restrictions of harsh environment at higher altitudes, thus triggering upward shifts of species ranges (Walther, Beißner & Burga 2005; Gottfried *et al.* 2012; Jump, Huang & Chou 2012). Species from warmer, lower vegetation belts took advantage of the improved growing conditions, in order to extend their leading edges to the subalpine belt at the expense of cold-adapted species already occurring there. The occurrence of typical montane-subalpine species, such as *Anthoxanthum alpinum, Vaccinium myrtillus* and *Alchemilla vulgaris,* considerably increased in all plots; in general, 14.3 species were newly found in plots belonging to the SES communities. Two species, *Ranunculus nemorosus* and *Briza media*, with the upper distributional limit in the montane belt, were detected in the plots for the first time. At the same time, we could not re-confirm the historical occurrences of 13.4 species; predominantly taxa with distribution in subalpine and alpine belts, such as *Ranunculus montanus, Nigritella nigra*, *Pedicularis rostrato-capitata,* decreased their frequencies. Also four species common for the high alpine zone (*Androsace obtusifolia*, *Minuartia verna*, *Oxytropis jacea* and *Salix retusa*) and present in the plots in historical surveys were not found in 2012. Although this species turnover did not affect species richness, it led to significant changes in the community structure.

Alpine vegetation responds to climatic changes by both shifting of species' elevational ranges and changes in cover of a species present in a community (Theurillat & Guisan 2001). Plant cover is a reliable indicator of environmental change in alpine habitats, due to high persistence of species (Schweingruber & Poschlod 2005; De Witte *et al.* 2012) and low rates of community dynamics (Körner 1999; Windmaißer & Reisch 2013); species cover normally does not vary much from year to year but shows a clear trend with increasing time intervals (Gottfried *et al.* 2012). The immigration of species from lower altitudes and increased environmental favorability for already present thermophilous species in the SES communities led to the increase of plant cover. During the last 50 years, vegetation cover of the subalpine grasslands increased on average in 23%. Similar to the biodiversity changes, there were distinct changes in frequencies of cold-adapted alpine species and thermophilous montane-subalpine taxa. Abundances of montane species, such as *Carlina acaulis*, *Leontodon hispidus* or *Helianthemun nummularium* clearly increased, whereas species with its main distribution in the alpine zone, such as *Agrostis alpina*, *Ranunculus alpestris* or *Primula auricula* considerably declined in the subalpine vegetation belt. Along with the biodiversity changes reported here, the structural vegetation changes may be considered as direct consequences of thermophilization, an Europe-wide process of shifts of effective physiological ranges of high mountain plant towards summits as a result of recent warming (Gottfried *et al.* 2012).

Environmental characteristics of alpine habitats, such as short vegetation periods, frequent frost-events and shallow nutrient-poor soils act as environmental filters, selecting for plants with traits suitable to survive under low-temperature stress and low resource acquisition (Diaz *et al.* 1999; Körner 1999; Spasojevic & Suding 2012). In terms of the LHS concept (Westoby 1998), alpine plants should have small leaves with large pools of non-structural carbon and high investments of non-structural carbon in (hemi)cellulose-rich sclerenchymatic tissues (i.e. low SLA values), which assist in coping with frost and wind (Atkin, Botman & Lambers 1996), short stature and low investments in seed mass (Körner 1999; Grime 2002; Poorter *et al.* 2009; De Frenne *et al.* 2013). Relaxation of the filter, due to recent warming, lead to changes in functional composition of alpine plant communities with a shift towards more resource acquisitive functional traits (Spasojevic *et al.* 2013). The RLQ analysis indicates that species which changed their frequency and abundance in the subalpine grasslands have different niche optima, resulting in changes in functional composition within the SES communities. During the last 50 years, species with traits that are advantageous under warmer conditions, such as high stature and heavier seeds, significantly increased their presences in the communities (20 out of 71 species). It is remarkable that the increase of warm-adapted species in the subalpine vegetation belt was more prominent at the slopes with north-western exposition. Apart from warmer temperatures for growth, species with high resource acquisition require better developed and more stable soils compared to alpine plants (Körner 1999); in the study region suitable soils are mainly found on slopes of northern and western expositions.

Changes in low alpine Caricetum firmae communities

The magnitude of alpine plant community response to warming strongly depends on available space and community composition (Holzinger *et al.* 2008; Erschbamer *et al.* 2009; Vittoz *et al.* 2009). The significant increase in species richness and plant cover observed in the low alpine vegetation belt (mean species richness increase in +6.4 species and +18% plant cover) might thus be explained by high levels of invasion susceptibility of the communities with ample available gaps in sparse vegetation, mainly structured by facilitative interactions. Whereas only few alpine species (e.g. *Helianthemum alpinum, Primula auricula* and *Salix retusa*) decreased their frequencies and abundances in the plots, a considerably higher number of species with montane and subalpine distribution, such as *Anthyllis vulneraria* and *Campanula scheuchzeri,* increased their frequencies and abundances. Also species already present in the communities in 1962, such as *Dryas octopetala, Carex firma* and *Ranunculus* *alpestris*, profited from warmer conditions and occupied available gaps; their abundance increased in the plots as well.

Along with gap availability, competition ability of present in a community species may significantly hamper or enhance immigration of newcomers (Choler, Michalet & Callaway 2001; Vittoz *et al.* 2009). Since short-stature, light-demanding and slow-growing cold-adapted alpine species are intolerant to competition (Körner 1999; Grime 2002; Gottfried *et al.* 2012), taller and fast-growing species from warmer, lower vegetation belts expanded their presence (both frequency and abundance) in the CFL communities at their costs. Similarly to the subalpine communities, the advance of species with traits suitable for higher resource acquisition were more prominent at north-western slopes, due to presence of deeper soils.

Changes in high alpine Caricetum firmae communities

It is generally accepted that especially upper alpine zone responds to warming by larger increases in species richness and abundances than adjacent belts, due to scattered vegetation providing ample space for invasion and spreading of plants (Pauli *et al.* 2007; Holzinger *et al.* 2008; Vittoz *et al.* 2008; Pauli *et al.* 2012; Wipf *et al.* 2013). However, in contrast to previous findings, we detected significant decrease in mean species richness (–4.7 species in comparison to 1961), due to a comparably strong reduction in frequency of high-altitude species, such as *Agrostis alpina*, *Minuartia sedoides* and *Oxytropis jacquinii* (on average 10.7 species were not found in the plots). Despite the apparent upward shifts of species distributional ranges in adjacent subalpine and low alpine vegetation belts, no species immigrated in the CFL communities from lower altitudes; the small increase of species frequency in the vegetation (on average +6 species) is explained by redistribution of species already present in 1962 among the plots. Changes in vegetation structure were also in contrast to previous studies. Despite temperature increase and presence of available gaps in the plots, the mean plant cover decreased from 75 to 60% during the last 50 years, with no clear trends in abundances changes among species with different altitudinal distribution. The direction of these changes, however, can be easily interpreted, when periglacial (Cannone, Sgorbati & Guglielmin 2007) and geomorphological (Pauli, Gottfried & Grabherr 2003) contexts of the study sites are considered. Sites with unstable surfaces, where permanent habitats are reduced by high frequency of disturbance events, due to weathering or permafrost melting, are characterized by stagnating or slightly decreasing species richness and reduced plant cover (Pauli, Gottfried & Grabherr 2003; Cannone, Sgorbati & Guglielmin 2007). The studied high alpine *Caricetum firmae* communities, especially the plots at the Funtenseetauern summit, are located close to the lower limits of permafrost occurrence in the Central Alps (Haeberli & Beniston 1998; Böckli, Nötzli & Gruber 2011). Therefore, the upward retreat of the permafrost zone caused by recent warming (Gruber, Hoelzle & Haeberli 2004; Gruber & Haeberli 2007; Böckli, Nötzli & Gruber

2011) could decrease slope stability and trigger disturbance events, such as debris flow and landslides. Additionally, frequent disturbances significantly decelerate processes of soil genesis in alpine environment, thus hampering the colonization of high altitudes by montane and subalpine species, since they demand developed soils with high water-retention capacity (Körner 1999; Erschbamer, Niederfriniger Schlag & Winkler 2008). Consequently, only species with root systems adapted to unstable substrates, which help them to anchor the plants, could establish and persist in the studies sites. Our results confirm this statement; species such as *Arctostaphylos alpinus*, *Dryas octopetala* or *Primula minima* not only maintaining their presence in the CFU communities, but also increasing their plant cover.

Changes in functional composition of the CFU communities were also contrasting to those found in subalpine and low alpine vegetation belts. The RLQ analysis detected an increased frequency of species with traits suitable for environments of high altitudes at the slopes of southern exposition and decreased abundances of fast-growing species with high SLA values during the last 50 years, a pattern opposite to expected vegetation response to recent warming. Again, hydrological context can aid to interpret the altered functional composition of the vegetation. Slopes of southern exposition receive more direct solar radiation which results in shorter duration of snow cover (Marke *et al.* 2013) and lower expression (or even absence) of permafrost (Haeberli &

Beniston 1998; Böckli, Nötzli & Gruber 2011). Although not measured in this study, we suggest that permafrost might degrade earlier on slopes exposed to the south than those of northern exposition which results in reduced frequency of disturbance events, thus allowing alpine plants from the local species pool to re-colonize the available gaps. Northern slopes, due to longer duration of snow cover, are preferably occupied by snow bed species which tend to have low-cost leaves with short life-span (i.e. high SLA values), in order to optimize carbon gain and thus overcome constraint of a shortened growing season (Choler 2005). Therefore, the decrease of snow cover duration, observed in last decades in the European Alps could negatively affect the snowbed species.

Chapter 6

Conclusions and perspectives

The present thesis shows that a functional approach has a big potential to improve our understanding of climate-induced vegetation changes at high altitudes. Therefore, it may be a also suitable tool to better predict the direction and magnitude of these changes under future climate scenarios.

In the first part of the thesis it was shown that plant functional traits could be used as direct correlates for explaining species occurrence along a temperature gradient. The strong relationship between SLA and temperature (Chapter 2) provides convincing evidence that this easy-measurable trait can be used as a predictor of species distribution ranges under changing temperature conditions. Slow-growing species that exhibit cell extension at lower temperature (low SLA values) will mainly be found in the cold part of temperature gradients, whereas fast-growing species with high temperature thresholds for cell extension (high SLA values) should be largely restricted to the sites exposed to higher temperatures.

However, the distribution and abundance of plant species are related not only to vegetative growth, but also to their reproductive success, due to high vulnerability of regenerative stages to environmental conditions. Mismatch between ambient temperature and temperature requirements of reproduction processes may, therefore, lead to low population fitness, due to limited or even unsuccessful production of seeds and germination. The strong relationships found between the temperature requirements of both pollen and seed germination (Chapters 3 and 4, respectively) and habitat temperature indicate that these two traits may be also responsible for the geographical boundaries of a species. Despite a strong correlation, these aspects were completely ignored in previous studies to explain and predict a plant's species distribution. We, therefore, argue that these patterns should be also tested in other climates and parts of the world.

Despite broad usage of functional approach in modern ecology, there are still several obstacles that may affect the strength of plant-climate relationships and thus the predictive power of functional traits. Therefore, in the second part of the thesis three important implications for correct application of the plant functional traits were considered:

1) Trait intraspecific variability. Due to plastic responses or genetic differentiation within a species, many traits may exhibit substantial level of intraspecific variation along environmental gradients (Albert *et al.* 2010; Lepš *et al.* 2011; Auger & Shipley 2013). Therefore, neglecting this source of variability in a functional analysis of floristic and vegetation changes, for example by considering trait values as being fixed, may lead to misestimation and even wrong results. Although the intraspecific

variability of specific leaf area (Chapter 2) were found to be relatively low (only 18% of studied species showed significant differences in their trait values along the temperature gradient), we argue that a test for a trait' variability at population level has to be included in all future studies on trait-climate interactions. Furthermore, the intraspecific variability of regenerative traits, such as seed and pollen germination, needs to be tested as well, since this aspect was not considered in the present thesis. Based on existing studies (Baskin & Baskin 1998; Kakani *et al.* 2005), we assume that there are some variability of seed and pollen temperature requirements at species level, especially among species with broad distributional ranges (e.g. Cavieres & Arroyo 2000). Additionally, temperature requirements of pollen germination and tube growth may also vary among species occurring at one site, due to their phenological differences. This aspect should be considered in future studies as well.

2) Complex effects of environment on trait variations along elevation gradients. Although elevation gradients are a perfect natural laboratory for investigating plant trait-temperature responses (Kőrner 2007), they encompass variations not only in atmospheric temperature, but also other environmental variables, such as soil fertility, water supply, irradiance, level of disturbance, etc (Canadell *et al.* 2007; Kőrner 2007). Because the majority of traits are intimately related to resource availability, the direct effect of temperature on a trait variability is often confounded by the

influence of these environmental factors (Ackerly *et al.* 2002; Garnier *et al.* 2004; Poorter *et al.* 2009). For example, the relationship between SLA and mean annual temperature at the community level (Chapter 2) was modified by the effects of disturbance and soil phosphorous content, explaining 9.8% and 8.9% of the modelled variation, respectively ($r^2=0.89$, $p<0.001$). Hence, these factors need to be considered when assessing the response of traits to temperature, by including in an analysis additional site characteristics, such as soil chemistry, soil depth, amount of precipitation, levels of disturbance, etc. Another solution for this problem could be an application of reproductive traits (e.g. pollen and seed germination; Chapter 3 and 4, respectively), variations of those are mainly ruled by temperature and only to lesser extent by other habitat characteristics.

The trait intraspecific variability along with the confounding effects of environment on trait variation suggest that trait values from existing trait databases, such as LEDA (Kleyer *et al.*, 2008) or Flora Indicativa (Landolt *et al.* 2010), should be used with caution, as they rarely indicate intraspecific variation or provide any climatic, habitat or edaphic information (Cordlandwehr *et al.* 2013). Moreover, the usage of the existing trait bases is limited by the fact that none of them consist comprehensive information on species occurring at high altitudes. Therefore, the development of an extended trait database for alpine flora, including comprehensive trait measurements together with site characteristics, therefore, is one of urgent task for alpine botany, which should be solved in the near future.

3) Phylogenetic bias. Since related species are likely to share similar attributes (Harvey & Pagel 1991; Lord, Westoby & Leishman 1995), trait variations along environmental gradient may be phylogenetically constrained (e.g. (Givnish 1987)). Although no trait-temperature relationships investigated in this thesis were found to be phylogenetically constrained, we recommend to test for phylogenetical signal in a trait data set in addition to common data processing procedures. Recently published dated phylogeny of a large European flora (Durka & Michalski 2012) along with modern phylogenetic methods (e.g. K-statistics (Blomberg, Garland & Ives 2003) implemented in the R statistical environment (*picante* package) (Kembel *et al.* 2010; R Development Core Team 2012) may considerably facilitate this task.

Despite its popularity and wide usage, the functional approach has hardly ever been applied in alpine botany to mechanistically understand vegetation changes of vegetation at high altitudes. Therefore, in the third part of the thesis we verified the functional approach as an additional tool to common floristic and phytosociological methods, in order to reveal mechanisms standing behind climate-induced vegetation changes in the Bavarian Alps in last 50 years. The results of the study indicated that species with traits that are advantageous under warmer conditions took advantage of increased environment favorability, as a consequence of recent warming, and increased their frequencies and abundances in subalpine and low alpine vegetation belts in the study region. In upper alpine vegetation belt, the functional traits could also help to explain the vegetation changes caused by permafrost degradation (as a result of the climatic changes). Although these results suggest that plant functional traits can be used as predictors of alpine vegetation response to climate warming, the analysis of the presented study still remains limited, since only three functional traits (SLA, canopy height and seed weight) were used. In order to increase the predictive strength of the functional analysis, we need to consider other key functional traits related to dispersal ability (e.g. dispersal distance, diasporous morphology), persistence (clonality, maximal plant age, seed banks), regeneration (seed reproduction, germination) and plant functioning under extreme low temperatures, such as frost-tolerance. It shows again, that more efforts in collecting data on functional traits of alpine plants are necessary. This is much more urgent than the development of new models on the effect of climate change of the flora. As shown in this thesis, traits strongly correlating to temperature were never taken into account in these models as well as other traits not measured in this thesis such as the dispersal ability of plants and the availability of long-distance dispersal vectors, respectively.

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Appendix

Chapter 2. Specific leaf area correlates with temperature: new evidence from trait variation at the population, species and community levels.

Appendix 1. Environmental characteristics of the collection sites.

					Water supply			Soil fertility					
N	**Plot**	**Altitude, m a.s.l.**	**Mean annual temperature, C°**	**Length of vegetation period (5°), days**	**Precipitation, mm a year**	**Soil moisture, m3 H20 per m3 soil**	**Soil depth, cm**	**Potassium, mg/kg of soil**	**Phosphorus, mg/kg of soil**	**Total carbon, g/kg of soil**	**Total nitrogen, g/kg of soil**	**Disturbance**	**Shadow**
1	**H2**	987	6.6	208	1748	0.57	18.3	45.3	11.7	151.3	8.9		
2	**H3**	1105	5.5	189	1338	0.32	26.1	47.2	4.5	148.0	7.0	+	
3	**H4**	1330	5.1	181	1631	0.42	11.4	97.5	16.6	192.8	14.3		
4	**H5**	1579	5.1	181	1631	0.70	13.5	35.6	20.6	184.9	13.8		+
5	**H6**	1808	4.6	170	1503	0.76	8.7	51.2	6.4	164.0	9.3		
6	**H7**	1984	3.9	131	1503	0.42	12.3	72.3	23.1	275.8	15.9		
7	**HO1**	841	6.6	208	1748	0.68	55.8	57.0	9.8	55.7	4.6		
8	**HO2**	1045	6.6	208	1748	0.66	11.8	43.4	12.5	110.0	9.8		
9	**HO3**	1333	5.5	188	1338	0.58	14.0	56.6	13.9	197.7	16.9		+
10	**HO4**	1552	5.1	181	1631	0.66	14.6	43.7	10.5	177.4	13.5		
11	**HO5**	1825	4.6	170	1503	0.54	8.3	51.4	11.0	157.7	4.9		
12	**T1**	699	7.9	224	1612	0.52	23,7	65.4	9.8	38.28	3.71		
13	**T2**	720	7.9	224	1612	0.50	48.90	66.12	12.36	43.27	2.75		
14	**T3**	656	7.9	224	1612	0.39	48.7	32.4	11.2	31.7	3.0		

Appendix 1. Environmental characteristics of the collection sites (continued).

N	Plot	Altitude, m a.s.l.	Mean annual temperature, C°	Length of vegetation period (5°), days	Water supply		Soil depth, cm	Soil fertility				Disturbance	Shadow
					Precipitation, mm a year	Soil moisture, m3 H20 per m3 soil		Potassium, mg/kg of soil	Phosphorus, mg/kg of soil	Total carbon, g/kg of soil	Total nitrogen, g/kg of soil		
15	**T4**	641	7.9	224	1612	0.36	20.70	38.12	4.22	77.09	4.96		
16	**T5**	746	7.9	224	1612	0.66	35.8	51.7	14.3	78.9	6.7		
17	**W2**	1017	5.7	191	1612	0.21	13.20	136.26	24.29	186.83	8.32	+	+
18	**W3**	1162	6.8	213	1503	0.78	16.4	67.4	40.6	205.1	7.8		
19	**W6**	1803	5.6	188	1503	0.36	15.15	51.20	6.82	174.61	11.26	+	
20	**W7**	2020	3.9	131	1503	0.59	19.0	65.7	24.8	136.2	8.8		
21	**WM 1-1**	766	7.9	224	1612	0.69	16.8	18.3	4.1	128.3	5.7		
22	**WM 1-2**	1245	5.7	181	1631	0.75	39.60	46.30	7.60	117.04	6.05		
23	**WM 1-3**	1423	5.7	181	1631	0.68	14.2	68.0	13.4	109.1	9.7		
24	**WM 1-4**	1773	5.1	181	1631	0.48	16.6	93.3	14.2	144.0	12.4		
25	**WM 1-5**	2032	3.9	131	1503	0.64	14.3	41.4	10.3	233.8	19.1		
26	**WM 2-1**	960	6.6	208	1748	0.51	10.10	86.22	30.33	230.34	10.17		
27	**WM 2-2**	1140	5.7	187	1612	0.53	8.9	91.7	20.2	179.8	10.2		
28	**WM 2-3**	1420	5.7	187	1612	0.50	14.15	165.86	33.52	247.98	13.51		
29	**WM 2-5**	1813	5.1	181	1631	0.64	25.40	32.60	10.91	60.70	5.80		

Appendix 2. Correlation matrix for environmental factors included in the final model as explanatory variables (Pearson correlation coefficient, r). Significant results are bold entries.

	Altitude	**Mean annual temperature**	**Disturbance**	**Shadow**	**Precipitation**	**Moisture**	**Soil depth**	**Potassium**	**Phosphorus**	**Nitrogen**
Altitude	-									
Mean annual temperature	**-0.92**	-								
Disturbance	0.01	-0.08	-							
Shadow	0.01	-0.12	0.26	-						
Precipitation	**-0.39**	0.36	-0.36	-0.21	-					
Moisture	0.14	-0.08	**-0.62**	-0.14	0.17	-				
Soil depth	**-0.5**	**0.48**	0.17	-0.19	0.2	0.04	-			
Potassium	0.05	-0.16	0.17	0.15	0.08	**-0.4**	-0.25	-		
Phosphorus	0.12	-0.13	-0.12	0.18	0.06	0.02	-0.28	**0.64**	-	
Nitrogen	**0.61**	**-0.63**	-0.04	0.3	-0.25	0.03	-0.61	0.26	0.28	-

Appendix 3. Community-weighted SLA values of the investigated plots.

Plot	Total number of species per plot	Number of species included in the study	Coverage of included species, %	Community mean SLA, mm2 mg-1	Community-weighted SLA, mm2 mg-1
1	79	50	81	22.78	21.30
2	93	69	87	20.68	17.90
3	105	75	88	22.30	19.99
4	77	52	92	23.98	22.55
5	66	57	99	20.71	17.12
6	61	54	90	20.32	13.98
7	99	43	60	24.19	24.89
8	70	47	78	24.37	25.04
9	59	45	91	24.97	22.07
10	79	61	91	21.29	18.74
11	72	53	93	20.31	16.94
12	53	12	73	26.77	25.31
13	64	41	52	25.56	25.26
14	67	42	87	25.43	24.89
15	70	42	68	24.57	23.55
16	64	45	89	25.92	25.01
17	71	47	42	20.04	19.56
18	74	56	83	23.37	25.44
19	80	59	68	21.60	17.70
20	69	53	85	20.35	14.66
21	78	41	76	24.26	22.17
22	80	58	65	23.48	23.75
23	83	59	82	23.11	22.07
24	91	67	93	22.40	20.56
25	39	33	99	19.52	11.15
26	91	50	64	25.38	26.15
27	106	78	58	24.14	24.51
28	105	62	70	23.27	22.07
29	84	65	87	21.32	20.66

Appendix 4. Collection sites of the studied species. MAT - mean annual temperature.

Species	**Collection site**	**Altitude, m a.s.l**	**MAT, °C**	**Coordinates**		**Collcection date**
				N	**E**	
Anemone nemorosa L.	NP Berchtesgaden, mountain pasture	1045	6.06	47;35.335	012;53.594	4/16/2011
Caltha palustris L.	NP Berchtesgaden, Hintersee lake, grassland	817	6.82	47;36.25	012;52.85	4/16/2011
Campanula alpina Jacq.	NP Berchtesgaden, alpine grassland	2051	2.74	47;32.708	012;49.887	6/21/2011
Campanula scheuchzeri Vill.	NP Berchtesgaden, subalpine grassland	1808	3.54	47;33.322	012;49.375	7/31/2010
Carex flacca Schreber	NP Berchtesgaden, to the west from Hintersee lake, grassland	987	6.25	47;34.481	012;48.058	5/10/2010
Gentiana asclepiadea L.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	1250	3.66	47;35.011	012;56.456	8/17/2010
Gentiana aspera Hegetschw.	NP Berchtesgaden, northern slope of Watzmann mountain, alpine grassland	2016	2.91	47;34.098	012;55.838	8/17/2010
Gentiana pannonica Scop.	NP Berchtesgaden, mountain pasture	1330	5.12	47;33.443	012;48.445	7/15/2010
Globularia cordifolia L.	Proteced area "Garchinger Heide", calcareous grassland	1773	8.03	48;17.328	011;38.940	5/8/2010
Helleborus niger L.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	450	6.34	47;35.429	012;56.643	4/16/2011

Appendix 4. Collection sites of the studied species (continued). MAT - mean annual temperature.

Species	Collection site	Altitude, m a.s.l	MAT, °C	Coordinates		Collcection date
Lychnis flos-cuculi L.	NP Berchtesgaden, mountain pasture	960	6.06	47;35.335	012;53.594	6/18/2011
Phyteuma orbiculare L.	NP Berchtesgaden, subalpine grassland	1045	3.54	47;33.322	012;49.375	7/15/2010
Plantago lanceolata L.	NP Berchtesgaden, mountain pasture	1808	6.06	47;35.335	012;53.594	6/18/2010
Primula minima L.	NP Berchtesgaden, alpine grassland	1045	2.74	47;32.708	012;49.887	5/25/2011
Primula veris L.	Proteced area "Garchinger Heide", calcareous grassland	2051	8.03	48;17.328	011;38.940	4/23/2010
Pulsatilla vulgaris Mill.	Proteced area "Garchinger Heide", calcareous grassland	450	8.03	48;17.328	011;38.940	4/23/2010
Ranunculus montanus Willd.	NP Berchtesgaden, subalpine grassland	450	4.23	47;34.985	012;52.903	6/4/2010
Ranunculus nemorosus DC.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	1600	6.34	47;35.429	012;56.643	6/17/2010
Ranunculus polyanthemophyllus W. Koch & Hess	NP Berchtesgaden, mountain pasture	960	6.74	47;36.245	012;52.847	5/20/2010
Soldanella alpina L.	NP Berchtesgaden, subalpine grassland	841	4.39	47;34.985	012;52.903	5/11/2010
Trollius europaeus L.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	1552	5.40	47;35.011	012;56.456	6/4/2010

Chapter 4. Initial temperature of seed germination as related to species occurrence along temperature gradient.

Appendix 5. List of the species collected for the germination experiments.

Species	Germination along temperature gradient
Aconitum tauricum Wulfen	-
Agrostis alpina Scop.	-
Androsace chamaejasme Wulf.	-
Anemone nemorosa L.	-
Anthericum ramosum L.	-
Arabis alpina L.	-
Bartsia alpina L.	-
Betonica alopecuros L.	-
Biscutella laevigata L.	-
Campanula alpina Jacq.	-
Campanula scheuchzeri Vill.	-
Carex atrata L. s.str.	-
Carex sempervirens Vill.	-
Chaerophyllum hirsutum L.	-
Clinopodium vulgare L.	-
Cynosurus cristatus L.	-
Dactylis glomerata L.	-
Elyna myosuroides (Vill.) Fritsch	-
Gentiana aspera (Hegetschw.) Dost. ex Skal.	-
Gentiana clusii Perr. et Song	-
Gentiana pannonica Scop.	-
Gentiana verna L.	-
Geranium sylvaticum L.	-
Helianthemum nummularium (L.) Mill.	-
Hieracium villosum Jacq.	-
Hieracium villosum Jacq.	-
Holcus lanatus L.	-
Homogyne alpina (L.) Cass	-

Appendix 5. List of the species collected for the germination experiments (continued).

Species	**Germination along temperature gradient**
Juncus monanthos Jacq.	-
Laserpitium siler L.	-
Linum catharticum L.	-
Myosotis alpestris F.W. Schm.	-
Parnassia palustris L.	-
Phleum hirsutum Honckey	-
Phleum phleoides (L.) H.Karst.	-
Phyteuma orbiculare L.	-
Potentilla erecta (L.) Rauschel	-
Primula auricula L.	-
Primula farinosa L.	-
Primula veris L.	-
Ranunculus acris L.	-
Rhinanthus glacialis Personnat	-
Rumex scutatus L.	-
Senecio abrotanifolius L. subsp. abrotanifolius	-
Trollius europaeus L.	-
Veratrum album L.	-
Achillea atrata L.	+
Achillea clavennae L.	+
Achillea millefolium L.	+
Adenostyles alliariae (Gouan) A. Kerner	+
Allium senescens subsp. montanum (Fr.) Holub	+
Anthoxanthum alpinum Å. Löve & D. Löve	+
Anthoxanthum odoratum L.	+
Arrhenatherum elatius (L.) Beauv. ex J. & C. Presl	+
Briza media L.	+
Buphthalmum salicifolium L.	+
Carduus defloratus L. s.str	+
Carex firma Host	+

Appendix 5. List of the species collected for the germination experiments (continued).

Species	Germination along temperature gradient
Carex flava s.str L.	+
Carlina acaulis L.	+
Centaurea jacea L.	+
Crepis biennis L.	+
Cynosurus cristatus L.	+
Deschampsia cespitosa (L.) P.B.	+
Dryas octopetala L.	+
Erigeron glabratus Bluff & Fingerh.	+
Geum rivale L.	+
Globularia cordifolia L.	+
Globularia nudicaulis L.	+
Horminum pyrenaicum L.	+
Hypochaeris radicata L.	+
Leontodon hispidus L. s.l.	+
Leucanthemum halleri (Suter) Duc.	+
Leucanthemum ircutianum Turcz.	+
Luzula campestris L.	+
Luzula sylvatica (Huds.) Gaud.	+
Origanum vulgare L.	+
Pimpinella major (L.) Hudson	+
Plantago lanceolata L.	+
Poa alpina L.	+
Primula minima L.	+
Ranunculus nemorosus DC.	+
Rumex alpinus L.	+
Scabiosa lucida Vill.	+
Sesleria albicans Kit. ex Schult.	+
Silene acaulis (L.) Jacq.	+
Silene dioica (L.) Clairv.	+
Silene nutans L.	+

Appendix 5. List of the species collected for the germination experiments (continued).

Species	Germination along temperature gradient
Silene vulgaris (Moench) Garcke	+
Soldanella alpina L.	+
Solidago virgaurea L.	+
Tofieldia calyculata (L.) Wahlenb	+
Trisetum flavescens (L.) Beauv.	+
Veronica fruticans Jacq.	+
Willemetia stipitata (Jacq.) Dalla Torre	+

Appendix 6. List of species from those the seeds were germinated along temperature gradient.

Species	**Collection site**	**Coordinates**		**Collcection date**	**Altitude, m a.s.l**
		N	**E**		
Achillea atrata L.	NP Berchtesgaden, alpine pasture	47;34.668	012;52.620	8/26/2010	1825
Achillea clavennae L.	NP Berchtesgaden, Wimbachtal valley, Trischübel, abandoned subalpine pasture	47;31.563	012;54.877	9/9/2009	1803
Achillea millefolium L.	Germany, Southern Bavaria	-	-	1/10/2009	300
Adenostyles alliariae (Gouan) A. Kerner	NP Berchtesgaden, abandoned mountain pasture	47;33.558	012;48.986	8/19/2009	1579
Allium montanum (Fr.) Holub	NP Berchtesgaden, Wimbachtal valley, Trischübel, abandoned subalpine pasture	47;31.563	012;54.877	9/16/2011	1803
Anthoxanthum alpinum Å. Löve & D. Löve	NP Berchtesgaden, Wimbachtal valley, Trischübel, alpine grassland	47;31.688	012;55.104	8/19/2010	2020
Anthoxanthum odoratum L.	NP Berchtesgaden, mountain pasture	47;35.335	012;53.594	7/14/2010	1045
Arrhenatherum elatius (L.) Beauv. ex J. & C. Presl	District Berchtesgaden, municipality Ramsau, lowland pasture	47;36.589	012;53.952	6/22/2011	714
Briza media L.	NP Berchtesgaden, mountain pasture	47;33.443	012;48.445	8/21/2009	1330
Buphthalmum salicifolium L.	Germany, Southern Bavaria	-	-	1/10/2009	400

Appendix 6. List of species from those the seeds were germinated along temperature gradient (continued).

Species	**Collection site**	**Coordinates**		**Collcection date**	**Altitude, m a.s.l**
		N	**E**		
Carlina acaulis L.	Germany, Southern Bavaria	-	-	1/10/2009	400
Carduus defloratus L. s.str	NP Berchtesgaden, Hirschwiesskopf mountain, alpine grassland	47 31.804	012 55.006	9/15/2011	2200
Carex firma Host	NP Berchtesgaden, northern slope of Watzmann mountain, alpine grassland	47;34.098	012;55.838	8/23/2009	2032
Carex flava s.str L.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	47;34.668	012;56.599	8/23/2009	1423
Centaurea jacea L.	NP Berchtesgaden, to the west from Hintersee lake, grassland	47;35.438	012;50.168	9/18/2010	817
Crepis biennis L.	District Berchtesgaden, municipality Ramsau, lowland pasture	47;36.589	012;53.952	7/1/2009	700
Cynosurus cristatus L.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	47;34.629	012.57.349	8/20/2009	960
Deschampsia cespitosa (L.) P.B.	NP Berchtesgaden, Wimbachtal valley, mountain grassland	47;33.194	012;53.078	9/5/2009	1162
Dryas octopetala L.	NP Berchtesgaden, northern slope of Watzmann mountain, alpine grassland	47;34.098	012;55.838	8/23/2009	2032

Appendix 6. List of species from those the seeds were germinated along temperature gradient (continued).

Species	**Collection site**	**Coordinates**		**Collcection date**	**Altitude, m a.s.l**
		N	**E**		
Erigeron glabratus Bluff & Fingerh.	NP Berchtesgaden, Wimbachtal valley, Trischübel, abandoned subalpine pasture	47;31.563	012;54.877	8/18/2010	1803
Geum rivale L.	District Berchtesgaden, municipality Ramsau, lowland pasture	47;36.522	012;54.723	7/1/2009	720
Globularia cordifolia L.	NP Berchtesgaden, mountain pasture	47;33.642	012;47.925	8/26/2010	1105
Globularia nudicaulis L.	NP Berchtesgaden, subalpine grassland	47;34.985	012;52.903	8/4/2010	1552
Horminum pyrenaicum L.	NP Berchtesgaden, Funtensee lake, grassland in the south from the lake	47;28.719	012;57.606	9/7/2009	1676
Hypochaeris radicata L.	Germany, Southern Bavaria	-	-	1/10/2009	400
Leontodon hispidus L. s.l.	NP Berchtesgaden, mountain pasture	47;35.335	012;53.594	8/4/2010	1045
Leucanthemum halleri (Suter) Duc.	NP Berchtesgaden, subalpine grassland	47;33.322	012;49.375	9/15/2010	1808
Leucanthemum ircutianum Turcz.	District Berchtesgaden, municipality Ramsau, lowland pasture	47;36.589	012;53.952	7/1/2009	714
Luzula campestris L.	NP Berchtesgaden, Funtensee lake, grassland in the south from the lake	47;29.353	012;56.800	8/23/2011	1600

Appendix 6. List of species from those the seeds were germinated along temperature gradient (continued).

Species	Collection site	Coordinates		Collcection date	Altitude, m a.s.l
		N	E		
Luzula sylvatica (Huds.) Gaud.	NP Berchtesgaden, mountain pasture	47;33.443	012;48.445	8/21/2009	1330
Origanum vulgare L.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	47;34.668	012;56.599	10/21/2011	1423
Pimpinella major (L.) Hudson	Germany, Southern Bavaria	-	-	1/10/2009	400
Plantago lanceolata L.	District Berchtesgaden, municipality Ramsau, lowland pasture	47;36.589	012;53.952	6/22/2011	714
Poa alpina L.	NP Berchtesgaden, subalpine grassland	47;33.322	012;49.375	8/1/2010	1808
Primula minima L.	NP Berchtesgaden, alpine grassland	47;33.130	012;49.796	9/15/2010	1984
Ranunculus nemorosus DC.	NP Berchtesgaden, to the west from Hintersee lake, grassland	47;34.481	012;48.058	8/21/2009	1045
Rumex alpinus L.	NP Berchtesgaden, abandoned mountain pasture	47;35.075	012;53.238	8/4/2010	1333
Scabiosa lucida Vill.	NP Berchtesgaden, Funtensee lake, grassland in the south from the lake	47;29.472	012;56.457	9/7/2009	1621
Sesleria albicans Kit. ex Schult.	NP Berchtesgaden, subalpine grassland	47;33.322	012;49.375	7/15/2010	1808

Appendix 6. List of species from those the seeds were germinated along temperature gradient (continued).

Species	**Collection site**	**Coordinates**		**Collcection date**	**Altitude, m a.s.l**
		N	**E**		
Silene acaulis (L.) Jacq.	NP Berchtesgaden, Wimbachtal valley, Trischübel, alpine grassland	47;31.688	012;55.104	8/24/2009	2020
Silene dioica (L.) Clairv.	Germany, Southern Bavaria	-	-	1/10/2009	400
Silene nutans L.	NP Berchtesgaden, to the west from Hintersee lake, grassland	47;34.481	012;48.058	9/2/2009	987
Silene vulgaris (Moench) Garcke	NP Berchtesgaden, Wimbachtal valley, mountain grassland	47;31.893	012;53.873	8/25/2009	1448
Soldanella alpina L.	NP Berchtesgaden, piedmont of Watzmann mountain, subalpine grassland	47;34.471	012;55.439	9/14/2010	1773
Solidago virgaurea L.	NP Berchtesgaden, piedmont of Watzmann mountain, mountain pasture	47;34.668	012;56.599	8/20/2009	1423
Tofieldia calyculata (L.) Wahlenb	NP Berchtesgaden, Funtensee lake, grassland in the south from the lake	47;29.472	012;56.457	9/8/2009	1621
Trisetum flavescens (L.) Beauv.	District Berchtesgaden, municipality Ramsau, lowland pasture	47;36.589	012;53.952	7/1/2009	714
Veronica fruticans Jacq.	NP Berchtesgaden, abandoned mountain pasture	47;33.558	012;48.986	9/1/2009	1579
Willemetia stipitata (Jacq.) Dalla Torre	NP Berchtesgaden, mountain pasture	47;35.335	012;53.594	7/9/2011	1045

Appendix 7. Correlation matrix for environmental factors included in the final model as explanatory variables (Pearson correlation coefficient, r). Significant results ($p < 0.05$) are bold entries.

				Ellenberg indicator value			**Seed**	
		Mean annual temperature	**Precipitation**	**Light**	**Moisture**	**Soil fertility**	**Coat thickness**	**Weight**
	Mean annual temperature	-						
	Precipitation	**-0.38**	-					
Ellenberg indicator value	**Light**	-0.26	-0.11	-				
	Moisture	0.04	0.03	-0.18	-			
	Soil fertility	**0.30**	-0.10	**-0.32**	0.26	-		
Seed	**Coat thickness**	0.16	0.07	-0.16	0.17	0.12	-	
	Weight	0.24	-0.1	-0.04	-0.19	0.14	**0.46**	-

Chapter 5. Altitude matters – contrasting effects of climate change on the vegetation development at different elevations in the Bavarian Alps

Appendix 8. Characteristics of the collection sites.

Summit	**Survey**	**Altitude, m a.s.l.**	**Aspect**	**Slope, degree**	**Coordinates**	
					N	E
Seslerio-Caricetum sempervirentis						
Hirschwiese	HW2	2030	SW	35	47°31.700′	012°54.991′
	HW3	2010	SW	30	47°31.691′	012°54.976′
	HW4	2000	SW	40	47°31.685′	012°54.976′
	HW5	1900	SW	30	47°31.624′	012°54.906′
	HW6	1800	SW	30	47°31.603′	012°54.906′
Schneiber	SR4	2300	SW	15	47°30.605	012°54.640′
	SR5	2230	SW	40	47°30.588	012°54.628′
Schneibstein	ST2	1950	W	25	47°33.944′	013°02.969′
	ST3	2000	N	35	47°33.915′	013°02.984′
	ST4	2050	SW	30	47°33.899′	013°02.964′
	ST5	2050	W	30	47°33.842′	013°03.000′
Viehkogel	VK4	2150	SE	10	47°29.293′	012°55.960′
	VK5	2140	SW	10	47°29.285′	012°55.937′
	VK6	2150	SW	15	47°29.292′	012°55.952′
Caricetum firmae lower stands						
Hohes Brett	HB1	2150	SE	10	47°35.019′	013°02.504′
	HB2	2150	SE	10	47°35.026′	013°02.528′
	HB3	2100	SE	20	47°34.992′	013°02.495′
	HB4	2100	SE	20	47°34.981′	013°02.487′
	HB5	2100	SE	20	47°36.023′	013°02.451′
	HB6	2130	SE	10	47°35.002′	013°02.547′

Appendix 8. Characteristics of the collection sites (continued).

Caricetum firmae lower stands						
Schneiber	SR1	2320	SW	5	47°30.644´	012°54.686´
	SR2	2320	SW	5	47°30.642´	012°54.670´
	SR3	2300	S	25	47°30.623	012°54.686´
Schneibstein	ST6	2170	S	20	47°33.655´	013°03.251´
	ST7	2180	S	20	47°33.664´	013°03.268´
Viehkogel	VK1	2140	E	35	47°29.293´	012°55.971´
	VK2	2130	SE	30	47°29.303´	012°55.982´
	VK3	2140	N	40	47°29.303´	012°55.960´
Caricetum firmae upper stands						
Funtenseetauern	FT1	2570	S	10	47°29.094´	012°58.626´
	FT3	2550	S	15	47°29.100´	012°58.525´
	FT4	2510	SW	35	47°29.137´	012°58.314´
	FT5	2500	SW	20	47°29.204´	012°58.208´
Teufelshorn	TH1	2320	W	30	47°29.364´	013°02.124´
	TH2	2310	W	25	47°29.370´	013°02.122´
	TH3	2350	W	25	47°29.358´	013°02.132´