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Ecology of alpine plants at their uppermost limit in the Himalayas

Ph.D. Thesis

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Annotation

This dissertation studies the key abiotic factors and plant characteristics that influence the performance of the highest growing herbaceous alpine plants in Ladakh, Western Himalayas, and examines vegetation changes likely associated with climate change. The study site, located in the Rupshu region of eastern Ladakh, hosts some of the world's highest-growing angiosperm plant species that occur above 6000 m a.s.l. The gentle slopes characteristic for the region, resulting in long elevational gradients, are particularly suitable for studying plant adaptations to the harsh dry and cold environment typical of whole Ladakh. The study presents novel insights into the causes of plant elevation limits and the direction of the vegetation shifts related to climate change at the very edge of the existence of angiosperms.

Declaration [in Czech]

I hereby declare that I am the author of this dissertation and that I have used only those sources and literature detailed in the list of references.

České Budějovice, 26.9.2022

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Zuzana Chlumská

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■ List of papers and author's contribution

The thesis is based on the following papers (listed chronologically):

- I. **Chlumská, Z.**, Janeček, Š., Doležal, J., 2014. How to preserve plant samples for carbohydrate analysis? Test of suitable methods applicable in remote areas. *Folia Geobotanica* 49, 1-15. (IF₂₀₁₄ = 1.778). *ZC participated in field sampling, sample preparations, enzymatic carbohydrate analyses, preparation of samples for HPAEC-PAD and wrote the manuscript with contribution of other authors. Her contribution was 80%.*
- II. Dvorský, M., **Chlumská, Z.**, Altman, J., Čapková, K., Řeháková, K., Macek, M., Kopecký, M., Liancourt, P., Doležal, J., 2016. Gardening in the zone of death: an experimental assessment of the absolute elevation limit of vascular plants. *Scientific Reports* 6, 2440 (IF₂₀₁₆ = 4.259). *ZC participated in the establishment and annual inspection of the experimental plots, plant measurements and co-authored the manuscript. Her contribution was 50%.*
- III. Doležal, J., Dvorský, M., Kopecký, M., Liancourt, P., Hiiesalu, I., Macek, M., Altman, J., **Chlumská, Z.**, Řeháková, K., Čapková, K., Borovec, J., Mudrak, O., Wild, J., Schweingruber, F., 2016. Vegetation dynamics at the upper elevational limit of vascular plants in Himalaya. *Scientific Reports* 6, 24881 (IF₂₀₁₆ = 4.259). *ZC participated in field sampling, sample preparations for nutrient and carbohydrate analyses, enzymatic carbohydrate analyses, sectioning of the samples for age analysis and revision of the manuscript. Her contribution was 40%.*
- IV. **Chlumská, Z.**, Liancourt, P., Hartmann, H., Bartoš, M., Altman, J., Dvorský, M., Hubáček, T., Borovec, J., Čapková, K., Kotlínek, M., Doležal, J., 2022. Species- and compound-specific dynamics of nonstructural carbohydrates toward the world's upper distribution of vascular plants. *Environmental and Experimental Botany* 201, 104985 (IF₂₀₂₁ = 6.028). *ZC participated in the conceptualization of the study, field sampling, sample preparations, enzymatic carbohydrate analyses, preparation of samples for HPAEC-PAD and wrote the manuscript with co-authors. Her contribution was 80%.*

■ ■ **Co-author agreement**

Miroslav Dvorský, the first author of paper II, and Jiří Doležal, the supervisor of this Ph.D. thesis and the senior author of all the papers mentioned above, fully acknowledge the stated contribution of Zuzana Chlumská to these manuscripts.

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Jiří Doležal

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Miroslav Dvorský

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GENERAL INTRODUCTION



Photo on previous page: subnival zone of the study site, on the high elevation plateau above Tsomoriri Lake, Rupshu region, Ladakh.

General Introduction

High mountains are synonymous in people's minds with a hostile landscape full of rocks and snow. Whoever dares to explore them closely will find a spectacular landscape occupied by a variety of colourful plants with interesting adaptations that allow them to live a contented life. There is no place in the world like the Himalayas where you can study the ecology of plants at their true upper limit of occurrence (Dvorský et al., 2015). As this unique environment is already impacted by ongoing climate change (Ballesteros-Cánovas et al., 2018; Das and Meher, 2019; Maurer et al., 2019) and intensified land use (e.g., Geneletti and Dawa, 2009; Gupta, 1978; Mishra et al., 2001; Rather et al., 2016), exploring the adaptations and limitations in plants and vegetation dynamics in alpine communities is essential for making predictions about their vulnerability or resilience, and perhaps about high-elevation ecosystems in general.

This dissertation studies the key abiotic factors and plant traits that influence the performance of the highest-growing herbaceous alpine plants in Ladakh, located in the Western Himalayas, and also examines vegetation changes that may be related to climate change. By monitoring permanent plots in the alpine and subnival zone, we studied the dynamics of high elevation communities at the upper elevational limit of plant distribution and the new colonization of unglaciated areas above this limit. We investigated changes in environmental conditions, species composition and abundance, but also in species characteristics (**Chapter I**). A manipulative experiment was used to determine the key abiotic factors and species characteristics that influence plant survival at high elevations, and also to explore the possibility of further upward movement of the vegetation (**Chapter II**). We also examined the importance of nonstructural carbohydrates (NSC) for the distributional limits of alpine plants (**Chapter IV**).

High-elevation vegetation dynamics and climate change

Alpine ecosystems with abruptly changing environmental conditions commonly belong to the biodiversity hotspots (Körner, 2021; Mutke and Barthlott, 2005; Rahbek et al., 2019; Testolin et al., 2021). However, they are predicted to be severely impacted by ongoing climate change. Glacier retreat due to rising temperatures is frequently observed in high-elevation ecosystems around the world and is commonly associated with changes in precipitation, especially diminishing snowfall. Rising temperatures are also expected, and have already been observed, to alter the precipitation regime and also intensify weather extremes, but long-term studies on this topic are generally lacking (Das and Meher, 2019; Gobiet et al., 2014; Hock et al., 2019; Pepin et al., 2022; Vuille et al., 2008). A less conspicuous, largely unexplored, but important element of high-mountain ecosystems is permafrost, the thawing of which will significantly affect substrate stability (Chiarle et al., 2021; Gruber et al., 2017; Ran et al., 2022). Taken together, temperature change poses a potentially great challenge to the mountain flora, fauna and humans and is therefore important to study (Adler et al., 2022; Chiarle et al., 2021; Huss et al., 2017; Milner et al., 2017; Mondoni et al., 2012; Vitasse et al., 2021; Yager et al., 2019).

High mountain ecosystems are characterized by large differences in abiotic conditions over small spatial distances (Scherrer and Körner, 2010), making any generalizations difficult. However, a growing number of studies are documenting changes in vegetation cover, including upward plant migrations due to rising temperatures and shifting permanent snowline (e.g. Hock et al., 2019). These changes have already been observed in Europe (e.g. Gottfried et al., 2012; Steinbauer et al., 2018), South America (Carilla et al., 2018; Cuesta et al., 2019; Sklenář et al., 2021), Australia (Auld et al., 2022; Verrall et al., 2021), and elsewhere (Angelo and Daehler, 2013). In Asia, studies from the Himalayas, home to the world's highest-occurring angiosperms (Birks, 2021), are still scant and often do not cover their highest locations (Anderson et al., 1995; Hamid et

al., 2020; Salick et al., 2019; Telwala et al., 2013), and recently only studies mainly from the high-elevation grasslands of the Tibetan Plateau have appeared (e.g., Huang et al., 2018; Liu et al., 2018; Verrall and Pickering, 2020).

Globally predicted scenarios commonly indicate future *thermophilization* of vegetation (De Frenne et al., 2013; Elmendorf et al., 2015; Feeley et al., 2020; Gottfried et al., 2012). In the mountains, subnival species are expected to be outcompeted by species from warmer lower elevations moving further towards melting glaciers, and may even become extinct when they reach bare, recently deglaciated peaks (Kulonen et al., 2018; Rumpf et al., 2018; Steinbauer et al., 2018). However, vegetation dynamics is a very complex process and many factors need to be taken into account (Corlett and Westcott, 2013) – species characteristics (Rixen et al., 2022), site-specific macro- and microclimatic conditions (Kulonen et al., 2018; Winkler et al., 2016), soil ecosystem (Hagedorn et al., 2019), biotic interactions (Alexander et al., 2015; Inouye, 2020), land use changes (Bhatta et al., 2018).

One of the ways to monitor vegetation changes is to regularly inspect areas above the current limit of vegetation distribution and search for new plants (Dvorský et al., 2015; Klimeš and Doležal, 2010). However, the summit terrain is usually very diverse and small plants can be overlooked, so it is convenient to determine the age of plants by anatomical analysis of annual growth rings, which is also possible for herbaceous species (Gärtner and Schweingruber, 2013). Another way to monitor vegetation dynamics is to establish permanent plots and install devices that monitor weather conditions (Wild et al., 2019). In addition to changes in floristic composition, an increasing number of studies also consider and observe changes in plant characteristics and their role in colonising new habitats (Matteodo et al., 2013; Rixen et al., 2022; Steinbauer et al., 2022).

The combination of these approaches can contribute to more accurate predictions of the future impact of climate change on alpine flora. Therefore, in our research, we used all these approaches to determine whether there is an increase in vegetation cover, species turnover and a shift in species

characteristics in the context of global climate change in the Western Himalayas (**Chapter I**). Our work follows the surveys carried out in 1997-2007 by our late colleague Leoš Klimeš, who examined literally every stone in the area and thus created a solid foundation for our current research.

Factors affecting the upper distribution of vascular plants

In light of the growing number of studies around the world tracking the upward shift in plant elevation ranges, the question arises as to what key factors influence the upper limits of their distribution. So far, we can document a steady state of colonisation and only hypothesize the role of increasing temperature and recently deglaciated habitable areas or changes in the precipitation regime in such a shift. The common factors that select for or limit upward movements of individual species given the high heterogeneity of alpine environments, or even the characteristics that enable plants to live at the highest elevations, are still widely debated (Körner, 2021).

Elevation is a multifactorial environmental gradient that primarily includes changes in air pressure, temperature, and solar radiation. However, the last two are also dependent on topography (slope and exposure) and cloud cover. Other factors important to plants vary regionally, such as precipitation, or are commonly more or less independent of elevation and vary less predictably along the gradient, such as geology, soil properties, and moisture (Körner, 2021).

Environmental conditions shape the appearance of alpine herbaceous communities and vegetation cover, e.g. by influencing the length of the growing season, soil nutrient availability and substrate stability (Körner, 2021). Especially in mountains, microtopography and thus microclimatic conditions vary considerably over a distance of several metres and can have an even greater influence on alpine vegetation than macroclimate (Litaor et al., 2002; Little et al., 2016; Scherrer and Körner, 2011, 2010).

Several limiting factors have been discussed as highly important for the establishment and survival of alpine plants. Low temperatures are among the most significant. Sub-zero temperatures during the dormant period, when plants are hardened, are usually not lethal (Körner, 2021; Neuner et al., 2020). Alpine plants seem to be well adapted to frost periods even during the growing season (Sierra-Almeida et al., 2009; Taschler and Neuner, 2004; but see Marcante et al., 2012). However, several studies have shown that plants can only thrive in places where the mean soil temperature during the growing season does not fall below 0 °C for at least four weeks, otherwise, they cannot reproduce, build up sufficient reserves, and generally develop normally (Körner, 2021, 2011; Körner et al., 2019; Nagelmüller et al., 2017, 2016).

Another important factor for plant growth and metabolism is the availability of nutrients in the substrate. Alpine soils at the highest elevations are usually infertile and generally poorly developed (Cicczazzo et al., 2016; Donhauser and Frey, 2018). In particular, soils are poor in nitrogen and its content depends on soil microbial activity and organic matter deposition, e.g. melting snow is a rich seasonal source (Hiltbrunner et al., 2005; Schulz et al., 2013). Other elements, such as phosphorus, are largely dependent on bedrock chemistry and weathering rates, which are also related to soil biological activity and temperature (Körner, 2021).

The depth and duration of snowpack is another critical factor in plant survival, as it protects plants from frost, but can as well shorten the growing season. It also affects the moisture content of the adjacent soil and increases the risk of frost heave, which can easily damage the root system or even uproot the plants. In addition to abiotic factors, specific plant characteristics may be more advantageous at higher elevations, such as the growth form enabling the plant to decouple from air temperature or the type of belowground organ and its resilience to soil movements (Körner, 2021). Diaspore type can also play a role in upward range shifts, e.g., plants with wind-dispersed seeds (with achenes or small seeds) may reach deglaciated areas faster than others (Erschbamer and Caccianiga, 2017; Zimmer et al., 2018).

The generally harsh conditions of the highest elevations inhabited by plants can be moderated by the presence of suitable *safe sites* with favourable conditions, particularly important for seedlings (Marcante et al., 2012). These can be larger stones, which buffer temperature fluctuations, by acting as heat traps, but also shade the adjacent soil and protect it from drying out; or patches of fertile soil (Ciccazzo et al., 2016; Schlag and Erschbamer, 2000). The high diversity of the alpine environment underlines the importance of in-situ measurements and experiments. Moreover, even adult, well-established plants can be destroyed by extreme weather events or disturbances that occur randomly over time, highlighting the need for long-term observational studies. Without knowledge of the crucial environmental factors and plant characteristics that make life at high elevations possible, it is very difficult to predict the future direction of cover and composition of alpine vegetation under climate change.

To reveal possible factors that are crucial for survival at high elevations but also enable or prevent alpine plants to move upward, we established a manipulative transplant experiment in the Western Himalayas, one of the places with the highest upper limit of continuous angiosperm plant cover in the world. The selected area (see below chapter **Study area**) is characterized by long elevation gradients due to the mild slopes of the peaks. It is particularly suitable for studies of the factors influencing the upper limit of species distribution, because the vegetation frontier in the subnival zone is not defined by a “hard” boundary, a permanent snowline, as for example in the Alps, but plants here remain several hundred meters below this line (Dvorský et al., 2016, 2015; Klimeš and Doležal, 2010). Experimental transplanting of plants to and above the current range limit is a way to answer questions about the key factors determining the current distribution and possible further colonization.

Methodological difficulties in the analysis of nonstructural carbohydrates in remote areas

Nonstructural carbohydrates are crucial compounds for plant metabolism. Their content is indicative of the carbon balance in plants and the effect of environmental factors on plant performance (Chapin et al., 1990; Martínez-Vilalta et al., 2016). However, researchers working on this topic have to cope with many methodological difficulties due to the multifunctionality of NSC and their high metabolic dynamics in plant tissues (Hartmann and Trumbore, 2016), e.g. loss by respiration or enzymatic conversion of higher polymers to simpler compounds.

Issues that need to be addressed prior to NSC analysis itself are how to preserve samples and inhibit metabolic processes (Landhäusser et al., 2018; Pelletier et al., 2010; Quentin et al., 2015). In remote areas without laboratory facilities nearby, such as high mountains, conventional methods (Landhäusser et al., 2018; Quentin et al., 2015) – freezing, microwaving, or even oven drying – are not possible. The first method requires heavy and bulky Dewar flasks filled with liquid nitrogen or dry ice, but the lids cannot be tightly sealed, so gases leak and can disappear in a short time. It is therefore not suitable for long sampling expeditions. The other method, microwaving, is a relatively new, fast and spreading way of stopping enzymes (Quentin et al., 2015) and the last method, oven drying, has been widely used in the past (e.g., Schappi and Korner, 1997; Wyka, 1999). However, even these methods are not suitable because ovens are heavy and require access to electricity.

All these obstacles led us to search for a more convenient method. We needed a method that would allow us to use lightweight and simple equipment to rapidly denature the enzymes that convert NSC and prevent sample degradation and preserve them for the time spent in the field, which in our case is usually about a month or more. We tested four extraction methods at different ethanol concentrations and temperatures and let the samples sit for

one month to simulate a delay before starting the NSC analyses. The rationale for using ethanol extraction was that it is a widely used pre-step in NSC analysis and therefore should not alter NSC concentrations and composition (Landhäusser et al., 2018). Finally, we compared the results with the conventional method of preservation in liquid nitrogen. The new method was then used in **Chapter IV**.

Dynamics of nonstructural carbohydrates in vascular plants at the upper limit of their distribution

NSC are very important compounds for plants. They serve as a carbon source for growth and as an energy reserve (Chapin et al., 1990; Martínez-Vilalta et al., 2016), and also help plants cope with stress by acting as osmolytes and cell stabilizers (for more functions see Hartmann and Trumbore, 2016). Photosynthesis is the sole source of carbon, and thus NSC, in plants. Carbon uptake by plants can be affected by adverse conditions in the high-elevation environment, like low temperatures or nutrient shortage (e.g., Ensminger et al., 2006; Fernández-Marín et al., 2020), as well as the relatively short period when assimilation is possible (Körner, 2011; Scholz et al., 2018). Several studies have been conducted on trees in high-elevation stands (treelines) and they have shown that the lack of a carbon source is probably not the cause of their upper distributional limits, since NSC in tissues do not decrease with elevation, and commonly even increase (Fajardo et al., 2012; Hoch and Körner, 2012, 2003; Shi et al., 2008).

Alpine herbs have also been found to have relatively high NSC content (Billings and Mooney, 1968; Hiltbrunner et al., 2021; Körner et al., 2019), but elevational patterns, particularly in belowground organs, are not usually studied. In treelines, it has been suggested that the higher NSC content is the result of passive accumulation, as low temperatures prevent trees from using NSC for growth. These studies have shown that assimilation is probably not a limiting process. Photosynthesis is robust to low temperatures and can operate

even at temperatures around 0 °C (Billings and Mooney, 1968; Ensminger et al., 2006; Körner and Diemer, 1987), but growth as a carbon sink is much more susceptible. Alvarez-Uria and Körner (2007) study on trees and Nagelmüller et al. (2017, 2016) studies on alpine herbs confirmed that meristem growth is suppressed at temperatures around 5 °C and ceases at temperatures below 2 °C (Nagelmüller et al., 2017, 2016, but see Körner et al., 2019).

Nevertheless, there are other studies that have attempted to explain the high NSC content in alpine plants. It is suggested that increased NSC content may also exist at the expense of carbon supply for growth (Li et al., 2018; Reyes-Bahamonde et al., 2021; Sala et al., 2012; Smith and Stitt, 2007). Wiley and Helliker (2012), proposed that prioritization of storage allocation may be an evolutionary response, at least in trees, to face harsh conditions at higher elevations that may cause more frequent episodes of carbon starvation. However, these hypotheses still await thorough exploration.

The role of NSC as stress mitigators (Ambroise et al., 2020; Livingston et al., 2009) may also be very important for alpine plants that experience freezing temperatures even during the growing season. For example, Hiltbrunner et al. (2021) and Möhl (2019) found relatively high levels of fructans and simple sugars in alpine plants and less starch, and these compounds seem to act in the process of cell stabilisation during freezing stress (Van den Ende and Valluru, 2008).

Most of the information we know about NSC in plants in relation to elevation is for trees at the alpine treelines. There is a great lack of information about the world's highest occurring angiosperms, the herbaceous species found in the Himalayas that can experience different conditions than trees. We thus attempted to fill this knowledge gap and conducted an experiment on 11 high-elevation plant species growing in the Western Himalayas to determine whether plants follow the general rule of increasing NSC observed primarily in trees towards their elevational limits. We also investigated and quantified various NSC compounds in these plants to determine whether the need for certain compounds potentially serving as cell protectants is greater at higher

elevations, as there is a general lack of information on this issue. For the experiment, we used the sample preservation method described in **Chapter III**.

Study area

The study area lies in the rain shadow of the Great Himalaya Range, in the so-called Trans-Himalayan region, specifically in the Rupshu region around Lake Tso Moriri (see Fig.). Average annual precipitation is low, less than 250 mm (Das and Meher, 2019; Leipe et al., 2014), and the permanent snowline, therefore, lies at very high elevations above 6000 m a. s. l. (Schmidt and Nüsser, 2017). Long-term climate trends from the study area measured *in situ* are missing. Data from this area and also from other regions of Ladakh or the Western Himalayas obtained from satellite images, models, and measured *in-situ* show increasing temperatures since the end of the last century and ambiguous results on precipitation trends (Chevuturi et al., 2018; Das and Meher, 2019; Shekhar et al., 2010; Shrestha et al., 2012).

Geomorphologically, the Rupshu region is an extension of the Tibetan Plateau and thus consists of high-elevation plateaus (ca 4500 m) with gently sloping peaks rising above 6000 m (van der Beek et al., 2009). The appearance of this area is different from well-explored mountain ranges such as the Alps or the Rockies, as the glaciated zone is usually not bounded by rock faces and has relatively well-developed soils extending to its edges. The Rupshu area thus has one of the highest distribution limits of continuous vegetation cover in the world (Birks, 2021; Dolezal et al., 2016).

Vegetation in the area is sparse, herbaceous species and small shrubs predominate, and trees are completely absent. Across the elevational gradient, there are three main habitats that differ in terms of soil water content, temperature, and thus the species composition of plants. In the lowest, warmest and driest elevations from about 4500-5300 m are alpine steppes with scattered, usually shrubby vegetation. Dense vegetation cover (grasslands) is bound to water bodies only. The alpine pastures extend above the steppes up

to 5600 m, the vegetation is denser due to the higher water content of the soil, but the conditions are cooler. Above 5600 m, there is a sparsely vegetated cold subnival belt (Dvorský et al., 2015, 2011).

Objectives and content of the thesis

Although there are a large number of studies monitoring changes in alpine vegetation in the context of global climate change, they do not focus on the world's highest growing plants and communities found in the Himalayas (Birks, 2021; Verrall and Pickering, 2020). Also, the main factors that enable or prevent alpine plants to live in such a harsh environment are not yet clear. However, this information is essential for predicting the future course of Himalayan vegetation and possibly alpine vegetation elsewhere.

Given these gaps in knowledge, this thesis examines: (i) temporal changes in elevational distribution, vegetation cover, species composition as well as characteristics of the highest occurring vascular plants in the Western Himalayas; (ii) the potential of these plants to move further up and the possible factors that determine their current elevational boundaries, in particular, low temperature, soil nutrient deficiencies, lack of suitable habitats, dispersal limitations and specific morphological and ecophysiological characteristics such as belowground organ type or growth form; (iii) the significance of NSC in forming distribution limits of Himalayan plants. To answer the last question, we had to (iv) find a new simple method using lightweight equipment to preserve NSC samples in remote areas.

Chapter I describes the vegetation changes at the world's upper limit of vascular plant distribution, changes in abiotic conditions and the main characteristics of the species that inhabit this area.

Chapter II presents a transplant experiment conducted in the subnival zone to assess the possibility of further upward migration, as well as the importance of

potential temperature, nutrient availability, dispersal and habitat limitations and the significance of specific species characteristics for upward movement.

Chapter III describes a new method of preserving samples for NSC analysis that is applicable in places where laboratory facilities are not available, and which was subsequently used in the study described in Chapter V.

Chapter IV describes the elevational dynamics of NSC in belowground organs of 11 Himalayan herbaceous plant species and its potential significance in the formation of their elevational distribution limits.

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CHAPTER I

Vegetation dynamics at the upper elevational limit of vascular plants in Himalaya

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Photo on previous page: view of the Lungser Kangri Peak glacier (6666 m a.s.l.), Rupshu region, eastern Ladakh.

Vegetation dynamics at the upper elevational limit of vascular plants in Himalaya

Abstract

A rapid warming in Himalayas is predicted to increase plant upper distributional limits, vegetation cover and abundance of species adapted to warmer climate. We explored these predictions in NW Himalayas, by revisiting uppermost plant populations after ten years (2003–2013), detailed monitoring of vegetation changes in permanent plots (2009–2012), and age analysis of plants growing from 5500 to 6150 m. Plant traits and microclimate variables were recorded to explain observed vegetation changes. The elevation limits of several species shifted up to 6150 m, about 150 vertical meters above the limit of continuous plant distribution. The plant age analysis corroborated the hypothesis of warming-driven uphill migration. However, the impact of warming interacts with increasing precipitation and physical disturbance. The extreme summer snowfall event in 2010 is likely responsible for substantial decrease in plant cover in both alpine and subnival vegetation and compositional shift towards species preferring wetter habitats. Simultaneous increase in summer temperature and precipitation caused rapid snow melt and, coupled with frequent night frosts, generated multiple freeze-thaw cycles detrimental to subnival plants. Our results suggest that plant species responses to ongoing climate change will not be unidirectional upward range shifts but rather multi-dimensional, species-specific and spatially variable.

Introduction

Cold biomes at high latitudes or high elevations are among the most dynamic systems on earth due to rising temperatures^{1,2}. Arcto-alpine biomes warm faster than global average², leading to increase in relative abundance of species adapted to higher temperature³, a process known as thermophilization⁴. Consequently, species which are narrowly specialized to cold habitats migrate upwards or experience local extinctions⁵. Ongoing distribution shifts^{6,7} and changes in plant cover, including the colonization of newly deglaciated areas⁸ are widely acknowledged⁹. Rapid warming is also changing plant phenology¹⁰, growth¹¹ and productivity¹².

Most of our knowledge about the recent vegetation changes in arcto-alpine systems comes from humid regions^{5,7,13-15}. While ongoing warming is likely to be the key driver of vegetation change in these cold and humid regions³, altered precipitation regime and its subsequent effect on soil water balance may be even more important in dry regions¹⁶⁻¹⁸. Changes in precipitation regime are often accompanied by an increase in extreme events² to which arid regions seem to be more vulnerable¹⁹. For instance, extreme precipitation in arid mountains can cause floods and landslides on poorly vegetated slopes²⁰ and the increased soil moisture together with associated frost heave can uproot plants²¹. Moreover, plant-plant interactions are likely to differ between humid and arid ecosystems^{22,23}. Because of the specific precipitation regime, arid regions may not undergo the processes of thermophilization generally described in cold biomes^{3,5}. The lack of knowledge about vegetation dynamics in response to climate change in cold and dry biomes is striking especially in dry continental Asia, where vascular plants grow in the highest elevations on earth. Ladakh, an arid mountainous region in the NW Himalayas, represents a unique region for studying vegetation dynamics and plant responses to climate change (Fig. 1 and Supplementary Fig. S1). Due to the dry continental climate, the mountains of Ladakh are often unglaciated up to 6200–6400 m. The unglaciated terrain together with dry climate allows plant species to grow in extreme

elevation even above 6000 m²⁴. Still, a relatively large and potentially colonizable area is found above the current upper distributional limit of vascular plants within this region. The mean temperatures in Ladakh have increased by 1.7 °C during the last century (Supplementary Fig. S2) and the warming accelerated in the 1990s²⁵ leading to rapid glacier retreat²⁶. Beside warming, Ladakh also experienced increasing precipitation, accompanied by more frequent extreme snowfall and storm events causing severe floods²⁷⁻²⁹.

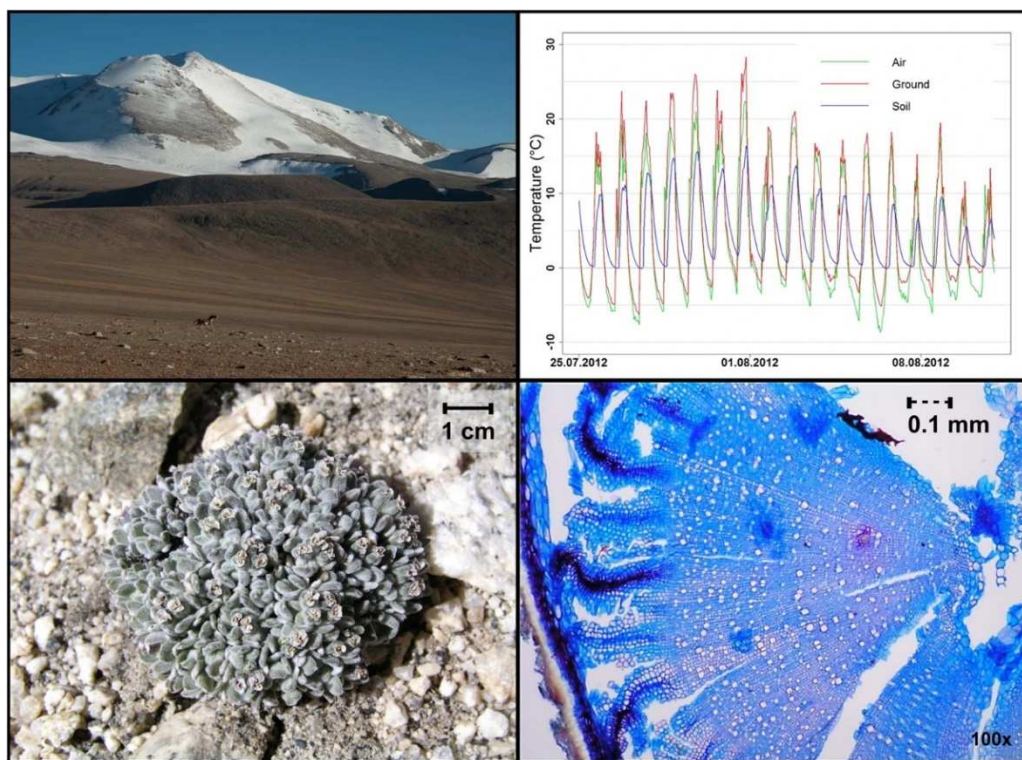


Figure 1. Study area in Eastern Ladakh, NW Himalayas. (A) Subnival belt around Chamser Kangri peak (6660 m a.s.l.). (B) Daily course of air, surface and soil temperatures in the peak of the vegetation season at 6150 m; the key for the survival of plants here is the rooting zone temperature during the growing season. From July to August, plants are able to withstand freezing air temperatures almost every night for 5–10 hours, but they never occur where the rooting zone temperature falls deeply below zero during the period of active growth. (C) The oldest plant (22 years) at 6150 m was an endemic species *Ladakiella klimesii*. (D) Its age was derived from ring counts on a 0.7 mm radius of root collar.

Plant responses to environmental changes, including climate warming and increased precipitation, can be predicted from their functional traits³⁰. Plant tissue nutrient content is one of the indicators of plant growth limitation at high elevations^{31,32}. High nutrient but also carbohydrate concentrations commonly found in alpine plants indicate the plant's inability to use the absorbed resources for growth (sink limitation) due to low temperatures restriction on enzymatic processes. Climate warming in the cold NW Himalayas can reduce sink limitation and increase relative abundance of faster growing taller species with optima at lower elevation steppes and semi-deserts. Contrary, increased precipitation and soil disturbance can support species from wet habitats such as springs and snowbeds, which are better adapted to repeated soil freezing and thawing. Hence, altered precipitation regime in the alpine and subnival zones of dry Himalayas can lead to the process of hygrophilization rather than thermophilization.

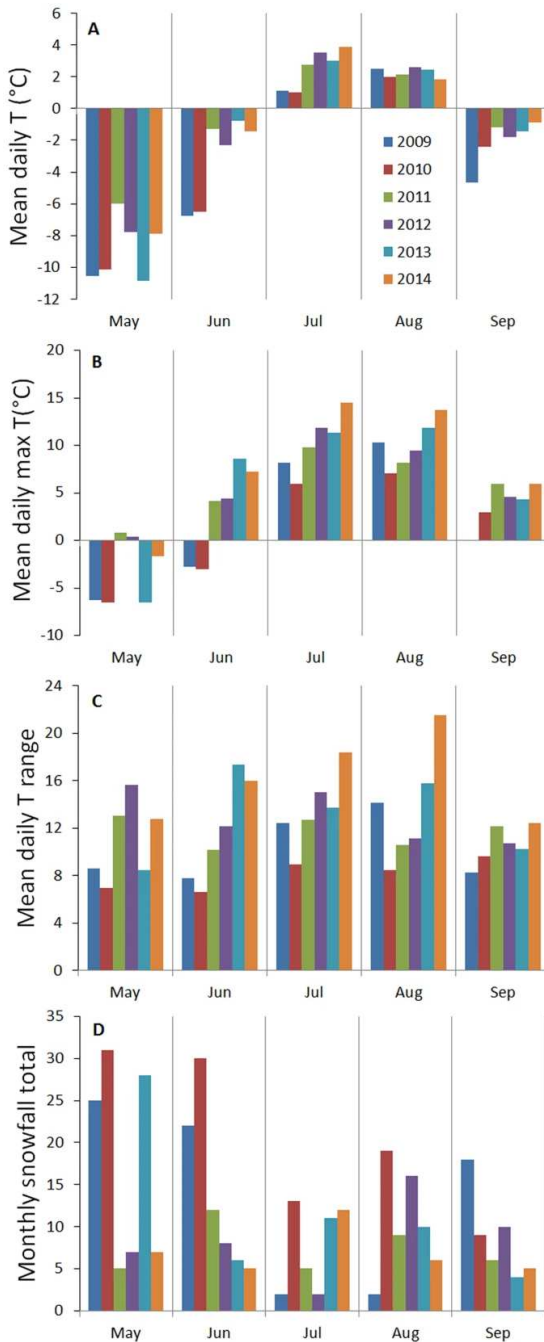
To explore upward plant migration and susceptibility of the cold and dry region of Ladakh to climate change, we (1) revisit an area extensively surveyed 10 years ago to find possible outpost populations above the previously recorded altitudinal upper limit; (2) re-survey permanent plots established in the alpine and the subnival belt after 4 years characterized by unprecedented warming and extreme summer precipitation; (3) characterize plant morphological and ecophysiological adaptation to evaluate the biological mechanisms of vegetation changes; (4) perform anatomical determination of plant age and growth rate. Plant age provides crucial proof of recent colonization and recent advances in anatomical assessment of distinct annual rings enables age determination, even in plants with a root collar thinner than 1 mm³³. Plants from cold unproductive environments tend to invest into longevity at the expense of fast growth, thus older plants are usually found at higher elevations³⁴. However, warming alleviates abiotic stress and reduces the need to invest into longevity, which in turn can trigger uphill plant migration^{4,35,36}. If this assumption is true, we can expect a negative relationship between elevation and plant age at the higher end of the elevational gradient. We

hypothesize that the ongoing climate warming has promoted uphill plant migration in this cold and dry region, so that absolute upper limits, species richness and cover of vascular plants increased in the past decade. If so, the species increasing in cover in the subnival belt should have syndromes of traits characteristic for lower elevation habitats. On the other hand, the evidences of thermophilization might be overridden by increased frequency of extreme snowfall events producing additional disturbance resulting in reduced species richness and cover.

Results

Climate changes

Overall, the period studied was characterized by warmer temperatures and extreme snowfall events (Supplementary Fig. S2). Long-term meteorological (1901–2014) records of temperature and precipitation in Ladakh show positive trends in mean winter, spring and fall temperature, with pronounced warming since the mid 1990s. The records also show a substantial increase in total precipitation and summer temperature since the mid 1990s. *In-situ* climate records from our study area for the period 2008–2014 confirm the general trend. There was a significant increase in mean daily maximum T for June (increase of 2.43 °C per year), July (+1.42 °C per year), August (+0.95 °C per year) and September (+1.02 °C per year; Fig. 2B and Supplementary Table S1). Similar trends were recorded for daily mean T in the summer months, except for August (Fig. 2A). The mean summer T (June to August) increased by 0.59 °C per year. The diurnal T range also increased (Fig. 2C). No major changes were recorded for winter month T, except for a cooling in February (–0.38 °C per year). The growing season length increased from 56 days in 2009 to 83 in 2010, 79 in 2012 and 75 in 2013. The summer 2010 was snowiest in past eight years (Fig. 2D).



Plant distributional limits and their upward shifts

During the first survey in 2001–2003, nine species reached 6000 m and above it: *Aphragmus oxycarpus*, *Draba altaica* and *Draba oreades* were found highest at 6000 m, *Ladakiella klimesii*, *Stellaria decumbens* and *Saussurea glacialis* at 6010 m, *Poa attenuata* and *Waldheimia tridactylites* at 6030 m and *Saussurea inversa* at 6060 m (Supplementary Figs S1 and S6). During the second survey in 2010–2013, there was one new species extending its range above 6000 m (*Saussurea gnaphalodes*). In total, the five species were found at higher elevations, the highest one being at 6150 m. These shifts extended the vertical range by 140 m in *Ladakiella klimesii*, 150 m in *Poa attenuata* and *Draba altaica*, 120 m in *Waldheimia tridactylites*, and 180 m in *Saussurea gnaphalodes*.

Figure 2. Climate conditions at the study area between January 2009 and September 2014. Mean and maximum monthly temperatures (T), the number of days with snow cover (snowfall total) and difference between maximum and minimum daily T (range) are shown.

Changes in diversity and composition in permanent plots

Total plant cover decreased both in the alpine (GLMM: chi-square = 94.4, $P < 0.001$,) and subnival (GLMM: chi-square = 35.7, $P < 0.001$, Fig. 3) vegetation between 2009 and 2012.

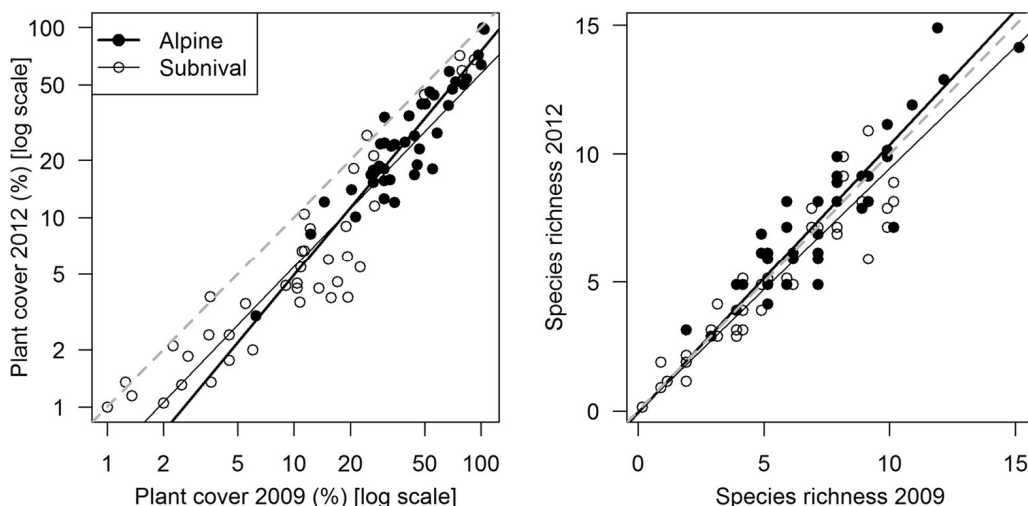


Figure 3. Changes in plant cover and species richness in the alpine and subnival vegetation between August 2009 and 2012. The dashed line represents the null hypothesis of no change in cover or richness between surveys and the solid lines represent a linear regression fitted to the alpine and subnival data.

Species richness showed no significant change in alpine vegetation (GLMM: chi-square = 3.607, $P = 0.998$, Supplementary Fig. S7) and marginally decreased in the subnival vegetation (GLMM: chi-square = 3.607, $P = 0.067$). The species composition changed between resurveys in both the alpine (standardized RDA, $F = 0.2$, $P = 0.002$, Supplementary Fig. S8) and subnival (the only significant statistic was non-standardized RDA, $F = 1.4$, $P = 0.008$) vegetation. In the subnival belt this was mostly due to changes in abundance and not floristic turnover.

The mean total cover per plot decreased from 46 to 31% in the alpine (mean relative change 35%) and from 17 to 10% (mean relative change 48%) in the subnival zone between the two sampling periods. 20 species (6 alpine and 14

subnival, Supplementary Table S2) significantly decreased in plant cover (mean relative change 40.1%), no species significantly increased in their cover. From 41 species recorded during the 2009 survey, three species were not found and five new species appeared during the 2012 survey; all of these losses and gains happened within the alpine vegetation.

Plant age distribution along elevational gradient

The alpine belt hosts species with a higher mean age while plants are usually younger and grow slower in the subnival belt (Fig. 4). The mean age of alpine and subnival plants was 20.5 and 11.4 years, respectively. The oldest plant individuals were *Potentilla pamirica* (70 years) and *Arenaria bryophylla* (53 years) in the alpine zone at around 5700 m.

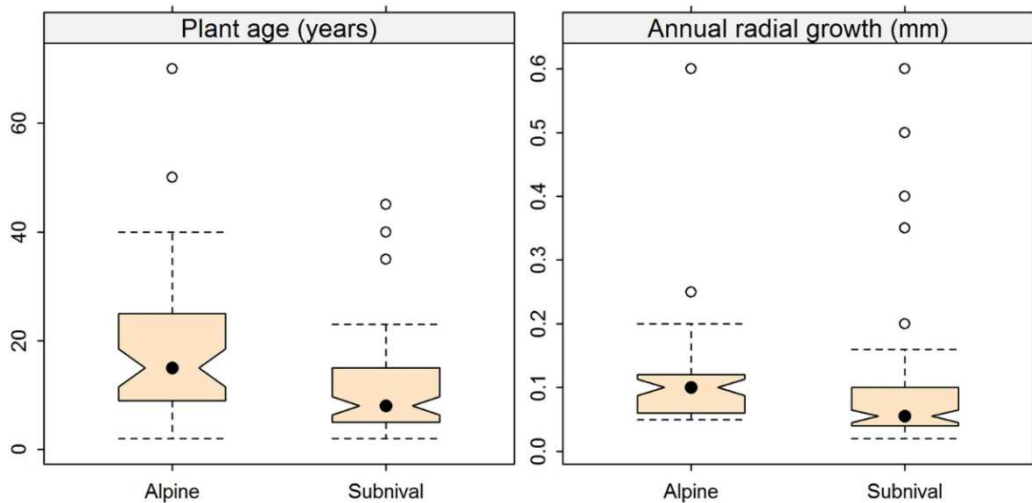


Figure 4. Difference in plant age and annual growth increment between alpine and subnival species. Non-overlapping notches in the boxes indicate the significance of between-group differences.

The most individuals found above 6000 m were established within the last decade (e.g. *D. altaica*, one specimen 8 year-old; *W. tridactylites*, three specimens 4, 4 and 9 years-old; *S. inversa*, three specimens 2, 6 and 6 years-old). The oldest plant individual at the highest site 6150 m was a 22 year-old endemic *L. klimesii* (Fig. 1D) and the two other specimens of this species were 8- and 16

years-old, indicating that some of outpost populations above 6000 m were established at least two decades ago.

Plant functional traits

Plant trait values differ significantly between alpine and subnival vegetation (Fig. 5, Table 1). 54% of the alpine species are clonal species with adventitious roots (spreading splitters) whereas subnival vegetation is dominated by small non-clonal cushion species (64% of the subnival species) with a main tap root producing belowground branches bearing aboveground leaf rosettes (spreading integrators). Subnival species are in average 21% shorter, 44% younger, grow

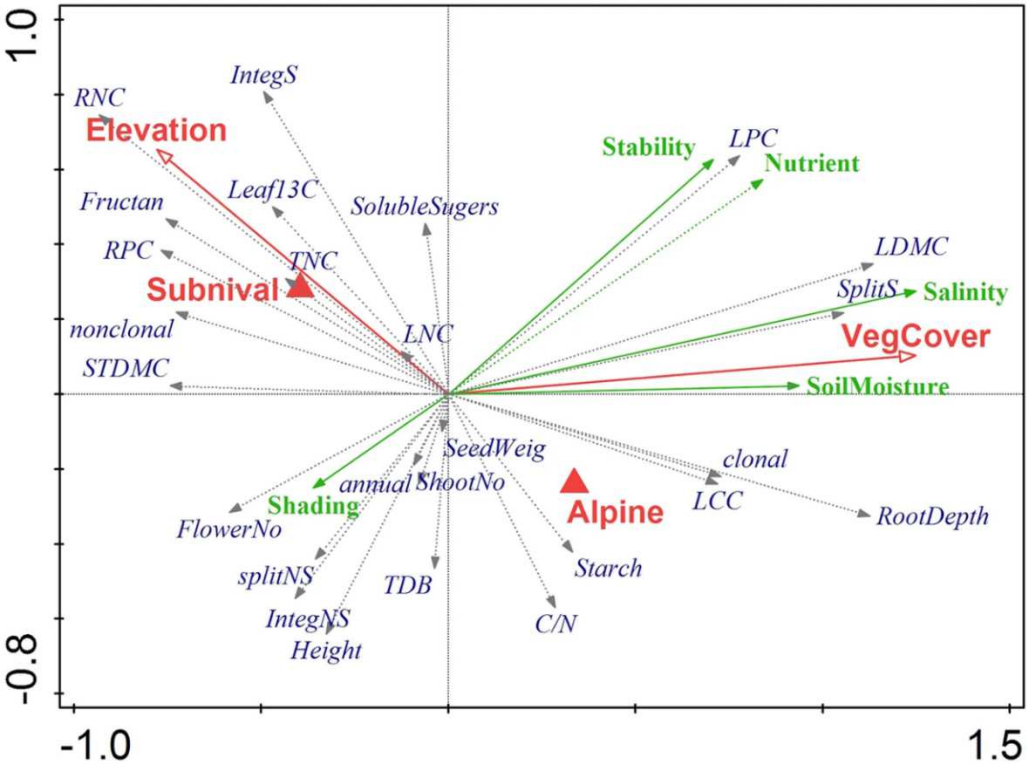


Figure 5. Redundancy analysis biplots (RDA) of community weighted mean values of plant functional traits (gray arrows) and habitat indicator values (green arrows) in relation to elevation, vegetation belt (alpine, subnival) and total vegetation cover (red color). The angles between arrows indicate correlations between variables (for explanation of trait and indicator values see Table 1). The environmental data explained 25.5% variability.

Table 1. Comparison of plant traits and indicator values between the alpine and subnival vegetation and the shift in trait community-weighted means between two sampling periods from 2009 to 2012. An upward or downward pointing arrow indicates an increase or decrease from the alpine to subnival, or temporal shift. Type I error estimates ^aP < 0.1, *P < 0.05, **P < 0.01, ***P < 0.001

Traits and habitat preferences	Abbreviation	Alpine09	Subnival09	P	Alpine_ diff_2009–2012	P	Subnival_ diff_2009–2012	P
Plant height (cm)	Height	14.00	11.00	↓**	0.204		1.058	↑*
Leaf nitrogen content (%)	LNC	2.26	2.42	↑*	–0.004		–0.007	
Leaf phosphorus content (%)	LPC	0.18	0.17		0.001		0.002	
Leaf carbon content (%)	LCC	42.84	41.59	↓***	0.169	↑***	0.414	↑*
Leaf carbon/nitrogen ratio	C:N	20.13	18.4	↓***	0.14		0.301	
Leaf δC ¹³ carbon (‰)	C ¹³	–26.21	–26.05		0.001		–0.008	
Leaf dry matter content (mg/g)	LDMC	176.63	131.84	↓**	7.14	↑*	1.608	
Stem dry matter content (mg/g)	STDMC	326.86	329.7		–14.39	↓**	52.84	↑ ^a
Root nitrogen content (%)	RNC	1.16	1.52	↑***	–0.032	↓***	–0.056	↓**
Root phosphorus content (%)	RPC	0.10	0.13	↑***	–0.003	↓***	–0.005	↓ ^a
Starch content (%)	Starch	2.48	2.00		0.02		–0.043	
Fructan content (%)	Fructan	2.72	4.78	↑***	–0.055		–0.492	↓*
Soluble carbohydrates (%)	SolubleSugar	4.02	4.38	↑*	–0.100		–0.047	
Total non-structural carbohydrates	TNC	9.19	11.17	↑**	–0.149		–0.581	
Seed weight (g)	SeedWeig	0.04	0.03		–0.003	↓*	0.001	
Root depth (cm)	RootD	14.18	8.94	↓***	0.439	↑*	–0.434	↓*
Number of aboveground tillers	ShootNo	18.02	15.83		–0.386		1.105	
Number of flowers	FlowerNo	7.36	7.24		–0.340		0.466	
Total dry biomass (g)	TDB	2.45	1.25	↓*	–0.018		–0.060	
Clonal plants with rhizomes (%)	Clonal	54	36	↓**	2.7	↑*	6.4	↑*
Non-clonal with tap-roots (%)	Nonclonal	46	64	↑**	–2.7	↓*	–6.4	↓*
Nonspreading integrators (%)	IntegNS	34	34		–0.6		–5.6	↓*
Nonspreading splitters (%)	SplitNS	34	31		0.8		5.5	↑ ^a
Spreading splitters (%)	SplitS	20	5	↓**	1.9	↑ ^a	0.9	
Spreading integrators (%)	IntegS	12	30	↑**	–0.022	↓*	–0.9	
Indicator value_moisture	Moisture	2.84	2.68	↓***	0.049	↑***	0.012	
Indicator value_stability	Stability	2.46	2.42		0.002		0.011	
Indicator value_salinity	Salinity	0.33	0.12	↓***	0.013	↑ ^a	0.003	
Indicator value_nutrient	Nutrient	0.98	0.98		0.007		0.005	
Indicator value_shading	Shading	0.09	0.09		–0.003		–0.006	
Elevational minima	Altmin	5056.6	5311.1	↑***	8.05		–29.9	↓*
Elevational optima	Alttop	5246.2	5534.4	↑***	–1.033		–24.2	↓*
Elevational maxima	Altmax	5708.9	5912.5	↑***	–1.469		–14.2	↓ ^a
Elevational range	Altrange	652.3	601.4	↓*	–9.520	↓ ^a	15.7	

25% slower, have 48% lower total dry biomass and 36.9% smaller rooting depth than alpine species (Table 1, Supplementary Fig. S9). Subnival species have significantly higher nitrogen concentration in their leaves (6.9%) and roots (30.6% respectively) as well as higher phosphorus concentration in roots (27.9%), but lower LDMC (25.3%), leaf carbon content (2.9%) and consequently lower leaf C:N ratio (8.6%) compared to alpine species. In addition, subnival species have 9.2% more soluble carbohydrates and 76.1% more fructans in their roots but do not differ in starch content compared to alpine species. Finally,

subnival communities are composed of plant species with an affinity for lower soil moisture and soil salinity compared to species from alpine communities (Table 1).

Shift in plant strategies in response to changing environmental conditions

Compositional changes observed in alpine and subnival vegetation from 2009 to 2012 were not ecologically random as evidenced from a shift in community-weighted mean trait values (Table 1). These changes in the CWM correspond to the decrease in abundance of the species with syndromes of highest elevation described in the previous section. The proportion of non-clonal species with a main tap root decreased between sampling periods (5.9% in alpine and 10.1% in subnival habitats), while that of clonal plants with adventitious roots increased (4.9 and 17.5%, respectively). This was accompanied by a significant decrease in root nitrogen (2.7 and 3.9%) and phosphorus (3.5 and 3.6%) concentrations. There was also a significant decrease in mean seed weight (7.1%) and increase in LDMC (4.4%) in alpine vegetation and increase in plant height (9.6%) in the subnival zone. Indicator values for soil moisture and salinity changed significantly between sampling periods in the alpine vegetation: species with an affinity towards wet and saline habitats (e.g. *Potentilla gelida*, *Halerpestes sarmentosa*, *Pegaeophyton scapiflorum*, *Koenigia islandica*) increased in cover relative to species from dry habitats (e.g. *D. altaica*, *P. pamirica*, *S. gnaphalodes*, *D. oreades*, Supplementary Fig. S8). In subnival vegetation, species with optima at higher elevations decreased in cover.

An alternative species-based approach confirmed that soil moisture indicator value is the best predictor of changes in species' cover in the alpine vegetation (Fig. 6). Species preferring drier surfaces were most prone to decline in their cover. The cover values of this group was further predicted by leaf $\delta^{13}\text{C}$ as a proxy of WUE, root phosphorus concentration as a proxy of growth rate and surface stability as a proxy of disturbance. Species preferring drier and stable

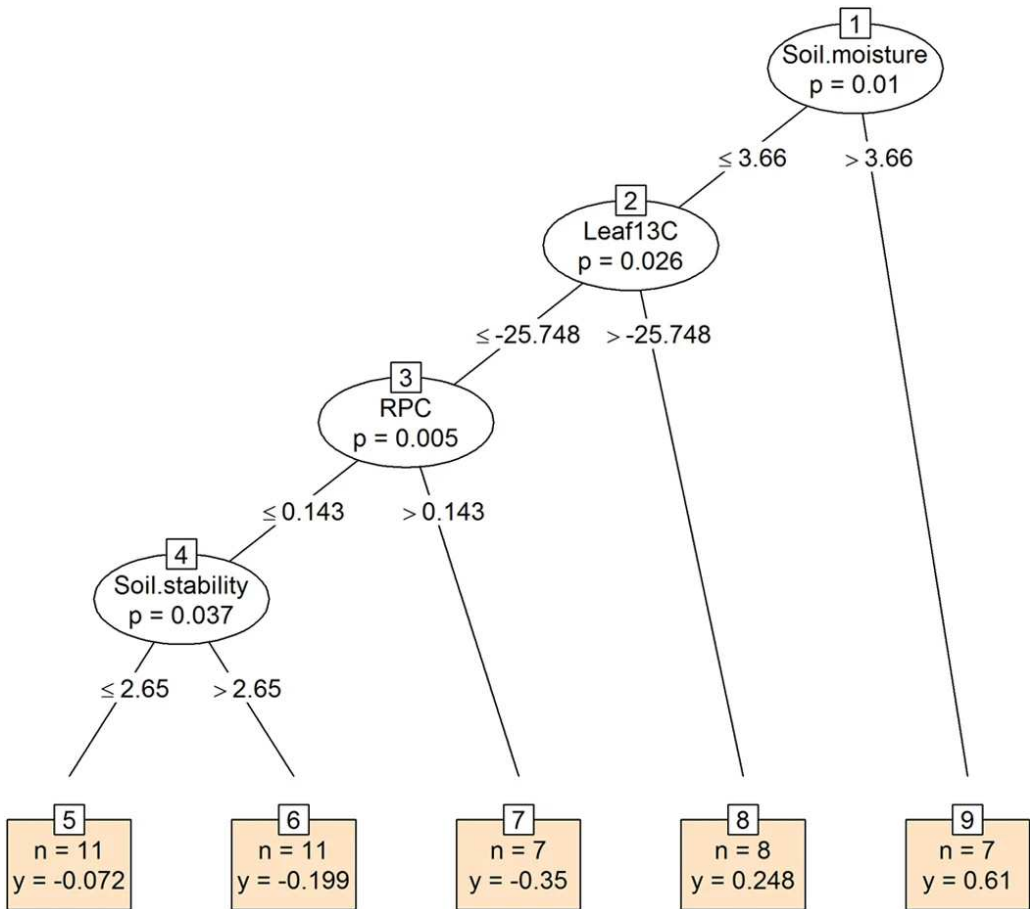


Figure 6. Life-history traits and ecological indicator values predicting species abundance changes in the alpine vegetation using the conditional inference tree. In each split of the tree, all species predictors are tested and the one that best discriminates between species is selected. The procedure goes on until no predictor significantly discriminates between species. The response variable (y) is the ordination score on the partial ordination first axis determined by a temporal change (boxes). Negative values indicate species declining over time and positive values indicate species expanding. Each split of the tree is described by the trait or indicator value used at the split (ovals), the permutation-based significance of the split (P -value) (ovals) and the threshold values at which the split occurs (for explanation of trait and indicator values see Table 1). The number of species (n) is given at each terminal node (box).

surfaces with lower $\delta^{13}\text{C}$ and higher RPC were more susceptible to decline in cover than species with higher $\delta^{13}\text{C}$ and faster growth preferring wetter and

naturally more disturbed habitats. In the subnival vegetation the only significant trait predicting changes in their abundance was space occupancy strategy (Supplementary Fig. S10). Species capable of clonal multiplication belonging to category of spreading splitters (*Carex sagaensis*) and spreading integrators (*Oxytropis platysema*, *W. tridactylites*, *Desideria pumila*) increased in cover, while non-clonal species with main tap roots belonging to category of non-spreading integrators (*A. oxycarpus*, *D. oreades*, *S. hypsipeta*, *L. klimesii*) or species with limited vegetative reproduction belonging to category of non-spreading splitters (*P. attenuata*, *Festuca non-coelestis*) were more prone to decrease in cover.

Discussion

The combination of site re-visitation, age determination and trait characterization did not provide evidence for thermophilization at the world upper limit for vascular plant life after an exceptionally warm decade. However, our results from the arid Himalayas do indicate the sensitivity of vegetation to the ongoing climate change. Unlike in the humid and cold high-altitude regions^{3,5}, the combination of warming and increased frequency of extreme snowfall events in the arid Himalayas²⁷ causes increasing disturbance events rather than purely ameliorating the level of stress. This results in a more complex pattern of vegetation changes than otherwise expected by the upward migration and thermofilization hypotheses^{7,37}.

Plant uphill migration and adaptive strategies

Accelerated warming in Eastern Ladakh over the past decade may have promoted the establishment of new populations at very high elevation, similarly as in other mountains^{5,13,14,38}. We discovered several outpost populations during the re-exploration of the Tso Moriri area above the upper vegetation limit determined 10 years before this study. While plant age analysis indicates that the highest outpost of *L. klimesii* was overlooked in the previous survey, *L.*

klimesii was the only plant species of the highest outpost that was over 10 years old. The other species have established within the warmer last decade. Also the younger age of the subnival species compared to the alpine species indicates recent establishments which coincide with the recent accelerated warming.

The observed upward plant migration was not ecologically random process as evidenced from plant life-history analyses³⁷. Subnival species that significantly extended their range by uphill shift evolved similar anatomical and ecophysiological adaptations despite differences in geographical origin, vertical distribution, thermal optima and phylogeny²⁴. Common features to all of them are the absence of fibers and the presence of thick-walled and small and short vessels (10–30 μm in diameter and 40–150 μm in length) which protect the plants against drought- and frost-induced cavitation and embolism³⁹. They are cushion-forming perennials with secondary growth, annual rings of semi-ring porosity and very small annual increments (ring width 0.03–0.05 mm) reflecting the harsh environmental conditions. These subnival species contain more nutrients and soluble carbohydrates and are capable of maximizing the rate of photosynthesis during short periods of favorable conditions, due to high foliar nitrogen content³¹ (Table 1 and Supplementary Fig. S9). Higher concentrations of simple sugars and sugar alcohols are thought to serve as osmoprotectants when drought or frost cause water deficit in the plant⁴⁰. These adaptations are crucial, because drought on sunny days and frost in cold nights are very common. Beside proper ecophysiological adaptations, the migrating species are anemochorous³⁷, such as *Saussurea* spp. from the Asteraceae family, which produces a large quantity of small wind-dispersed seeds with an attached feathery pappus, or *Draba* spp. and *A. oxycarpus* from the Brassicaceae family which have extremely light seeds²⁴.

While proper organismal adaptations to freezing temperatures and drought are necessary prerequisite for upward plant migration⁴¹, it remains question if there are some shared physiological constraints beyond which plants cannot move³¹. There is increasing evidence that the coldest places on Earth with angiosperm plant life are determined by a growing season (defined by a daily

mean $>0^{\circ}\text{C}$ in the rooting zone) of at least 50 days, with seasonal mean root zone temperature around $2\text{--}3^{\circ}\text{C}$ ³². This could be considered as the universal minimum for persistent angiosperm life³², although further evidence is needed. Klimes and Dolezal⁴² found the upper limit of plant life at 6030 m on the western slope of the Chalung mountains in 2002 (10 km north of our study site), formed by *Poa attenuata* and *Waldheimia tridactylites*; in-situ measurements over 2002–2003 showed a 47 day growing season with mean root zone (-5 cm) T of only 1.7°C . Unfortunately, the existence of plant life at this precisely georeferenced site was not confirmed during the revisitation in August 2013 (Dolezal, unpublished data), indicating the transient character of outpost populations in extreme elevations. Interestingly, what is common for the uppermost plant populations for which in-situ measured climate data are available (Mt Chalung 6030 m and Mt Shukule 6150 m), despite the difference in absolute elevations and seasonal mean T (1.7°C vs 2.8°C), is at least four continuous weeks without frost in the rooting zone. This suggests that the temperature of the rooting zone during the growing season is more important for vascular plant life at its upper limit than other commonly used climate measures. The four-year records from 6150 m indicate that freezing of the soil is more critical for vascular plants than frost above the ground^{31,32}. While the air temperature during the short growing season drops below zero every night for 5–10 hours, repeatedly to about -5°C (Supplementary Fig. S11), the soil temperature remains above zero (Fig. 1B). Plants more easily cope with freezing air than soil because of the differences between shoot epidermis and root rhizodermis; the root rhizodermis represents a less sufficient barrier against ice-nucleation upon contact with extrinsic soil ice masses, especially in juvenile plants⁴³. Most of the upper subnival species in Ladakh have a thick bark, large cortex and a xylem with much living parenchyma with high soluble carbohydrate concentrations (Fig. 1 and Supplementary Fig. S9) as protection against night frost during the short summer season.

Thermophilization or hygrophilization?

In addition to increasing temperatures during the past decade, the other aspect of climate change in the region, the extreme snow fall events²⁷ and the subsequent frost heave (Supplementary Fig. S5), when the soil is saturated with water, could pose a serious challenge to vascular plants adapted to arid soils of cold alpine deserts^{31,43}. The extreme snow fall events during the study period coincide with the decrease in vegetation cover (increase in bare ground) by roughly one third, for both alpine and subnival communities. Such short term climate events can have long-lasting effects⁴⁴. While increased temperature could promote plant regeneration, the very slow growth rate common to species in this system, is unlikely to compensate for the increased frequency and intensity of the disturbance.

Trait analyses of the re-surveyed alpine and subnival vegetation did not provide support for thermophilization processes. Evidence for thermophilization would have been indicated by an increase in the abundance of species with trait syndromes and elevational optima common for thermophilous or xeric species from habitats of lower elevations, such as steppes and deserts⁴⁵. Instead, the vegetation composition in permanent plots in the alpine belt shifted towards clonal species preferring wetter habitats (Table 1). We observed an increase in hygrophytes, a phylogenetically diverse group of plants growing in permanently or temporarily wet soils that are sometimes waterlogged and frozen almost every night on the surface during summer. These were not only species with high affinity to soil moisture, but also those with higher leaf δC^{13} (higher WUE) and lower P concentration. This indicates that increasing moisture is not the only cause of the observed vegetation change but also increased disturbance due to repeated freeze-thaw cycles, which plants with higher WUE can better cope with. The higher P content is characteristic of slow growing alpine specialists^{24,31,32}. Most prone to decline are therefore species with lower WUE and slow growth not capable of clonal

multiplication occupying drier and stable surfaces, i.e. the common species at the highest elevations in arid NW Himalayas.

Hence, the climate change in this arid region, involving warming and increasing precipitation, may not lead to the process of thermophilization as observed in cold and humid regions³, but rather to hygrophilization by increasing species from azonal wet habitats having otherwise small spatial extent in these arid mountains. We attribute the vegetation shift to the adverse effects of increasing daily maximum temperatures, which in combination with snowfall during growing season, creates freeze-thaw cycles. Unprecedented warming recorded in the period 2008–2012, with an increase in the mean daily maximum T by 2.4 °C and 1.4 °C per year for June and July, respectively, causes faster melting of snow instead of slow sublimation. The warming is accompanied by increasing diurnal fluctuations (Fig. 2C) as minimum daily T did not change significantly over time and hence the night frosts are still present. All this contributes to the recurrence of freeze-thaw actions (Supplementary Fig. S6). It is therefore the simultaneous increase in summer temperature and precipitation that can be detrimental for populations at the upper limit of plant distribution, in particular those with a single main root and adapted to dry and stable substrates. The abrupt damage to the alpine and subnival vegetation, which took place after snowfall summer 2010, will hardly be compensated soon because of the extremely slow growth in extreme elevations. Therefore, the impact of the increasing frequency of extreme precipitation events may, in the longer term, suppress the vegetation due to its immediate effect, while the positive effects of warming are relatively slow.

Resilience to climate change

Dvorsky *et al.*²⁴ found that the generalist species with wide vertical ranges and optima in the alpine and steppe zone are more abundant in subnival flora of Ladakh than high-elevation specialists (see also Supplementary Figs S1 and S6). Subnival specialists with narrow elevational ranges represent 42% of the flora. Also among nine species forming uppermost populations above 6000 m are five

generalists (*Aphragmus oxycarpus*, *Saussurea glacialis*, *Waldheimia tridactylites*, *Poa attenuata*, and *Draba altaica*) having optima at around 5100 m elevation and vertical ranges over 2000 meters long. The fact that subnival flora is composed of both specialist and generalist species suggests its relatively high resilience to climate change including both warming and increased frost disturbances in this region. Continued warming could potentially lead to thermophilization and increased abundance of steppic and alpine plants causing subnival specialists to decline at the alpine-nival ecotone. This was recently seen in wet lower mountains^{5,35} where subnival specialists already reached to mountain tops and hence cannot escape competition from alpine species by uphill migration. This is an unlikely scenario in the arid NW Himalayas because the vast unglaciated areas between 6000–6400 m are available for range extension of the subnival species^{24,42,45}. Complete extinction of subnival flora due to increasing precipitation and frost-disturbance is also unlikely given the high proportion of generalists among subnival taxa with their source populations at lower elevations capable of recolonizing sink habitats above.

Conclusion

Knowledge of vegetation dynamics at the altitudinal limit of vascular plants is crucial for understanding plant migration processes, physiological limits and diversity changes^{4,5,15}. Here, we explore the vegetation dynamics in the extreme altitudes of arid Ladakh, a rapidly warming region in the NW Himalayas, to test the ‘upward migration and thermophilization’ hypotheses, which predict an increase in absolute species distributional limits, vegetation cover and abundance of species adapted to warmer climate. We re-surveyed outpost populations after 10 years and permanent plots after 4 years from an area between 5500 and 6150 m.a.s.l. In addition, we determined the age and various functional and ecophysiological traits related to adaptations of plants to extreme altitude. We discovered several outpost populations during the re-exploration of the area above the upper vegetation limit determined 10 years

before this study, supporting the upward migration hypothesis, with five subnival species extending their vertical range by 120–180 m, reaching an elevation of 6150 m. The plant age analysis corroborated the hypothesis of warming-driven uphill shift. However, the force of warming pushing plants upwards has been recently offset by extreme snowfall events as those in summer 2010. Vegetation resurvey of permanent plots revealed a substantial decrease in plant cover and compositional shift towards species preferring wetter habitats. Declining species have lower water-use efficiency, slow growth and main taproot and are typical for the dry and cold NW Himalayas. Contrary, species whose cover increased were mostly clonal hygrophytes growing typically in wet soils. These vegetation changes are likely due to increasing soil moisture content and physical disturbance. Simultaneous increase in summer temperature and precipitation which causes rapid snow melt and, coupled with frequent night frosts, generates frost heaves detrimental to subnival plants. These relatively short-term disturbance episodes can have long-lasting effects because slow growth rate of high-altitude plants is unlikely to compensate for such severe disturbance. By combining vegetation resurvey with anatomical age determination, we conclude that climate change in the arid Himalayas produces a complex pattern of plant responses involving uphill migration due to warming and vegetation decline due to increasing precipitation and soil disturbance. Actual effects of climate change in arid mountains are thus more complex than usually thought.

Material and Methods

The study was conducted on the southwest spur of the Tibetan Plateau in Eastern Ladakh (Fig. 1A), Jammu and Kashmir State, India, on a high-altitude plateau 15 km East of Tso Moriri Lake (33.1 N, 78.2 E) at elevations ranging from 5500 to 6150 m, between Chalung, Shukule and Chamser Kangri Peaks. The region is arid (Leh: 115 mm/yr, 3514 m, ca. 170 km NW of the study region, Gar: 54 mm/yr, 4232 m, ca. 160 km SE of the study region), because most of the

summer monsoon precipitation is blocked by the main Himalaya Range. In the foothill desert areas around 3000–4500 m elevation, annual rainfall does not even reach 100 mm; at elevations above 4500 m precipitation tends to increase. Precipitation falling in summer above 5000 m is mostly snow. Winter precipitation is rather erratic and the snow layer is usually thin⁴⁶. Soils have a coarse-grained structure, with a high percentage of large gravel, low water and organic matter contents, high pH (7–8) and relatively high concentrations of total N and P⁴⁷.

In situ climate measurements

To gain detailed information about inter-annual climate variation in the region, we set up a network of automatic HOBO and TOMST[®] TMS microclimatic stations recording air and soil temperature (T), air relative humidity (RH) and soil moisture at hourly intervals (Fig. 2). Microclimatic stations have been in place since August 2008 to September 2014 and were placed at ~100 m vertical intervals. The climate records show that the growing season length, defined here as the number of days where the soil temperature constantly exceeded 0°C (frost-free days), shortens by 9.6 days per 100 m (from 90 to 28 frost-free days), while the mean temperature of the growing season decreases on average by 0.39°C per 100 m elevation (from 5.2 to 2.8°C) along the studied vertical gradient (5500–6150 m) encompassing the alpine and subnival vegetation. Soil moisture content, averaged for the entire growing season, is generally low and varies from about 4 to 15%, with the highest values recorded at around 5700 m.

To monitor the local snow conditions, an automatic PlantCam[™] camera (www.wingscapes.com) was placed on a slope facing the permanent plots and climate loggers in the period 2011–2012, taking a photo every day at 10 a.m. The close relationship between measured air relative humidity and local snow conditions allow us to calculate frequency of snow events over the whole measurement period (for details see Dvorsky *et al.*²⁴). Continuous snow cover usually occurs from February until April but with great differences between years (Fig. 2D). There were 86 days with snow cover in a late winter period

(March–May) in 2009, 92 in 2010, 54 in 2011, 41 in 2012 and 89 in 2013 (Supplementary Fig. S4). Temperature and relative humidity fluctuations were low in the April–May periods of 2010 and 2013 (Supplementary Fig. S3), which coincided with unusually deep snow covers, followed by a warm June and July. This led to high soil water content and increased the risk of frost heave (Supplementary Fig. S5), with mean daily minimum T ranging from -2.4 to -10.5 °C (absolute daily minimum T for June–July range from -4.3 to -18.1 °C), while the mean daily maxima reached $+14.5$ °C (Fig. 2B). The summer 2010 was snowiest in past eight years, there were 26 snowfall days in 2009 between June to August, 62 in 2010, 26 in 2011, 26 in 2012 and 27 in 2013.

Assessment of upward migration and thermophilization

To explore the possible uphill migration over the last decade, we examined the elevational distribution of subnival plant species in the study region (Supplementary Fig. S1). We compared plant distribution data collected in 2001–2003^{42,45,48} with our recent distributional data collected in the same region in 2011–2014^{24,49}. Depending on habitat diversity and species richness, individual localities were searched for about 30 minutes to one day. The elevation of individual floristic records was measured with an altimeter (Thommen, Switzerland) and Garmin GPS and stored in a floristic database of Ladakh^{42,48}.

To explore possible thermophilization of species assemblages, we analyzed temporal changes in species composition, cover and richness in 80 permanent plots. These plots were established within the alpine (41 plots, 5500–5800 m) and subnival zone (39 plots, 5800–6030 m) in 2009, on a gentle west-facing slope (3–5 degrees of inclination) and similar exposition. Each plot was 1 m² in size and divided into one hundred 10 × 10 cm subplots with all species of vascular plants and their cover being recorded. In 2012, we resurveyed all these plots following the same sampling protocol after a period of rapid climate warming (Fig. 2). The subnival zone was distinguished by a floristically-based nivality index²⁴, the absence of steppe species and by the presence of subnival

specialists. Also, the plots with cover >50% were all recorded below 5800 m (in most cases contained clonal graminoids such as *Carex sagaensis* and *Kobresia pygmaea*), marking transition between the alpine and subnival zone^{24,45}.

Plant age and growth determination

To evaluate the age and growth of plants in high elevation communities, we measured 426 individual specimens of 67 herbaceous species collected in the alpine (43 species) and subnival belt (24 species) in August–September 2012–2014 (Fig. 1A). Due to dry soils in the study region perennial plants often have deep tap-roots⁵⁰ and distinct annual ring increments. All monocot species (21%) were excluded because they are not suitable for herbochronological analysis. Three to four adult undamaged individuals of each species were excavated and the washed roots placed in plastic bags containing ethanol until further analysis. The samples were analyzed in the Swiss Federal Research Institute WSL, in Birmensdorf, Switzerland by a method developed by Gärtner and Schweingruber⁵¹. Transverse, tangential and radial sections were cut from all studied individuals using a sliding microtome. Microscopic images of optimal ring width sequences were made for counting of the consecutive number of rings (Fig. 1D) and measuring the width of annual increments⁵¹. Plant ages were determined by ring counting in the oldest sections in the transition between the hypocotyl and the primary root (root collar). The possibility of non-transparent rings due to inappreciable growth in extremely cold or snowy summer cannot be excluded, therefore the numbers of counted rings represent minimum plant ages.

Plant functional traits

To assess the biological mechanisms indicative of thermophilization, we measured several morphological and ecophysiological traits (see Table 1 for abbreviations of the traits). These were: plant height, total dry biomass, number of flowers and shoots, seed weight, rooting depth, leaf dry matter content, leaf carbon, nitrogen and phosphorus concentrations, leaf $\delta^{13}\text{C}$, root nitrogen and

phosphorus concentrations and the content and composition of nonstructural carbohydrates. Starch and fructans were analyzed using colorimetric methods. Soluble sugars, i.e. raffinose family oligosaccharides, sugar alcohols and simple sugars were quantified using high-performance anion exchange chromatography with pulsed amperometric detection (for details see Chlumská *et al.*⁵² and Supplementary Fig. S9). Leaf δC^{13} , which measures the ratio of ^{13}C over ^{12}C (%), is an integrated, long-term measure of the ratio between internal and ambient CO_2 concentrations (C_i/C_a) that reflects the intrinsic water use efficiency (WUE) of plants⁵³.

The traits were measured in a minimum of 10 individuals (up to 50 for some species) from various elevations within the studied region and the average values were calculated per species. In addition to quantitative traits, each species was classified as clonal or non-clonal based on the type of belowground organs (rhizomes with adventitious roots versus primary tap-roots) and further into four space occupancy strategies, based on the rate of lateral spread (spreading—more than 10 cm per year; non-spreading—less than 10 cm per year) and persistence of connections between ramets (splitters—plants producing adventitious roots with main root decaying; integrators—plants not producing adventitious roots and/or with perennial main root⁵⁰).

Habitat preference

In addition to plant traits, we included information on high-altitude species' ecological optima in order to predict species' response to climate change. Species' optima on five environmental gradients were derived from vegetation composition of 369 plots (each 100 m²) sampled in a stratified design to cover major vegetation types over the study area^{24,45,48}. On each sampled plot, five environmental variables were estimated: (1) soil/substrate stability: 1—unstable (scree, dunes, solifluction soils), 2—partly stable (grasslands, steppes), 3—stable (rocky crevices, *Kobresia pygmaea* mats); (2) light availability: 1—shaded (gorges, shaded rocky crevices, walls of stream banks), 2—partially shaded (dense vegetation cover), 3—full light (sparse vegetation cover); (3) soil moisture: 1—dry

(substrate usually without visible traces of water), 2–mesic, 3–wet (water level regularly but transiently above soil surface), 4–permanent surface water; (4) soil fertility: 1–low (semi-deserts, steppes), 2–medium (alpine meadows), 3–high (stables, animal resting places); (5) soil salinity: 1–no salt deposits on soil surface, 2–salt deposits scarce, 3–salts forming a continuous crust. From these data, indicator values of a species were calculated as arithmetical means of indicator values in individual plots in which that species was recorded, weighed by the logarithm of its cover.

To obtain a robust estimate of the elevational optima of the species, we calculated response curves fitted with HOF models⁵⁴. Species response curves were derived from 4,150 vegetation plots (each 100 m × 100 m) sampled over the entire Ladakh between 1999 and 2014 (Supplementary Figs S1 and S6). The dataset contain more than 122,000 records of occurrence of vascular plant species along exceptional elevational gradient from 2800 m to 6150 m^{24,45,48}. Plant names follow Klimes and Dickore⁵⁵.

Physico-chemical characterization of the soil

To characterize physico-chemical parameters of soils, 50 g was taken as a composite of 5 subsamples from randomly selected points within each plot in 2009. The samples were air dried for 24 h on aluminum plate, after than placed into sterile polypropylene bags (Nasco Whirl-Pak®) and transported to the laboratory for analyses. In the laboratory, the soil samples were oven-dried at 100 °C, grinded in a mortar and sieved to 2 mm fraction after the removal of roots. Major cations (Ca^{+2} , Mg^{+2} , K^{+} and Na^{+}) as well as nitrogen and phosphorus were measured in all soil samples. Cations were quantified through atomic absorption spectroscopy (AAS) using SpectrAA 640 (Varian Techtron) at the Analytical laboratory of Institute of Botany, Czech Republic. Ammonia, nitrate and total nitrogen were determined colourimetrically after Kjeldahl mineralization using automatic FIAstar 5010 Analyzer (Tecator). Phosphorus was determined colourimetrically after digestion in HClO_4 using SHIMADZU UV - 1650PC spectrophotometer. Other physico-chemical data were also

measured: pH, water content, organic matter content (OM) and texture (fraction of particles >0.5 mm in diameter). The alpine soils had significantly higher total N, P, Ca concentrations and organic matter content and lower texture than subnival soils (Supplementary Fig. S12).

Statistical analysis

To assess temporal changes in the plot-level vegetation records (number of vascular plant species, total plant cover, individual species' frequency and cover) between the surveys (2009 vs 2012) and between the habitats (alpine vs subnival) and their interactions, we used generalized linear mixed-effect models (GLMM^{56,57}). The tests were based on the restricted maximum likelihood (REML) approach approximated by chi-square distribution. The statistical significance was assessed by computing Bayesian highest probability (HPD) intervals using Markov chain Monte Carlo simulations (999 permutation in each test).

To explore shifts in species composition, we performed multivariate ordination analysis RDA, separately for the alpine and subnival belts. The variance partitioning procedure was performed in RDA with explanatory variables (year) and co-variables (elevation, plot identity) to remove their effects and to obtain a net effect of the year. To assess whether compositional change is driven by uniform shift among species or rather by differential species responses, RDA was performed both with and without standardization by sample norm⁵⁸. Compositional differences in plant assemblages were tested by 999 permutations.

To explore further the ecological causes for the species temporal responses, we used plant functional traits and habitat indicator values in explaining the vegetation changes. Two approaches were employed: (1) *plot-based analysis using trait averages* (weighted by log-transformed species cover abundance; hereafter *CWM*, community weighted means), compared between two sampling periods using GLMM as described above. Because obtained results reflect mainly the features of dominant species, we also used an alternative approach which places individual taxa into focus instead of individual plots: (2)

species-based analysis of the trait-environment relation was a two-step analysis, in which the temporal change of individual species was first quantified (by scores on the first ordination axis of partial RDA, with the year as the only explanatory variable and elevation and plot identity as covariables) and then those scores were related to the traits and habitat indicator values of individual species using a *conditional inference trees*⁵⁹. Conditional inference trees has several advantages over other classification and regression trees algorithms, including the statistical testing of each split through permutation, no need for problematic pruning of over-fitted trees and no selection bias towards variables with many possible splits or missing values.

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Author information

Contributions

J.D., M.D. and M.K. designed the study. All authors collected field data. J.D., M.K., M.D., M.M. and J.W. performed statistical analyses. F.H.S., Z.C., J.A. and J.D. performed age and growth analysis. Z.C. and J.B. performed carbohydrate analyses. J.D., P.L., M.D. and M.K. wrote the first draft of the manuscript and all authors contributed substantially to revisions.

Ethics declarations

Competing interests

The authors declare no competing financial interests.

Supplementary information

Supplementary Table S1. Changes in temperature (daily max, mean, range T) and snowfall totals (number of days with snow cover) from January 2009 and September 2014 at 5900 m in Eastern Ladakh, NW Himalayas. Linear regression parameters (slope and intercept) with explained variance R^2 and corresponding type I error estimate P are given.

Mean daily maximum temperature		R^2	P
JAN:	$y = -0.0521x + 86.689$	0.006	0.888
FEB:	$y = -0.3825x + 751.82$	0.537	0.098
MAR:	$y = -0.4119x + 814.58$	0.404	0.175
APR:	$y = -0.0831x + 157.66$	0.003	0.925
MAY:	$y = 0.655x - 1320.9$	0.119	0.503
JUN:	$y = 2.4281x - 4881$	0.848	0.009
JUL:	$y = 1.4269x - 2859.9$	0.791	0.018
AUG:	$y = 0.9502x - 1901.3$	0.642	0.030
SEP:	$y = 1.0213x - 2050.4$	0.736	0.014
OCT:	$y = 1.2472x - 2512.2$	0.445	0.148
NOV:	$y = 0.6029x - 1222$	0.161	0.430
DEC:	$y = 0.4399x - 898.96$	0.218	0.350
Mean daily temperature			
JAN:	$y = -0.4803x + 943.73$	0.307	0.254
FEB:	$y = -0.3852x + 753.92$	0.670	0.046
MAR:	$y = -0.1389x + 261.3$	0.308	0.253
APR:	$y = -0.1452x + 278.15$	0.041	0.699
MAY:	$y = 0.2697x - 551.29$	0.068	0.618
JUN:	$y = 1.2272x - 2471.8$	0.709	0.035
JUL:	$y = 0.5909x - 1186$	0.821	0.013
AUG:	$y = 0.1172x - 233.65$	0.184	0.337
SEP:	$y = 0.69x - 1390$	0.795	0.007
OCT:	$y = 1.0713x - 2165$	0.562	0.085
NOV:	$y = 0.2028x - 423.9$	0.052	0.665
DEC:	$y = 0.0861x - 192.97$	0.047	0.680
Diurnal temperature range			
JAN:	$y = 0.7819x - 1565.2$	0.512	0.109
FEB:	$y = 0.0896x - 174.15$	0.128	0.486
MAR:	$y = -0.4835x + 980.46$	0.255	0.307
APR:	$y = 0.2162x - 426.23$	0.013	0.830
MAY:	$y = 0.8x - 1598.2$	0.195	0.381
JUN:	$y = 2.1471x - 4307.1$	0.858	0.007
JUL:	$y = 1.3286x - 2658.9$	0.632	0.058
AUG:	$y = 1.487x - 2977.4$	0.506	0.073
SEP:	$y = 0.5792x - 1154.5$	0.615	0.036
OCT:	$y = 0.2194x - 429.53$	0.066	0.624
NOV:	$y = 0.6566x - 1308.9$	0.261	0.300
DEC:	$y = 0.5948x - 1186.3$	0.393	0.183
Snowfall total			

HIGH-ELEVATION VEGETATION DYNAMICS AND CLIMATE CHANGE

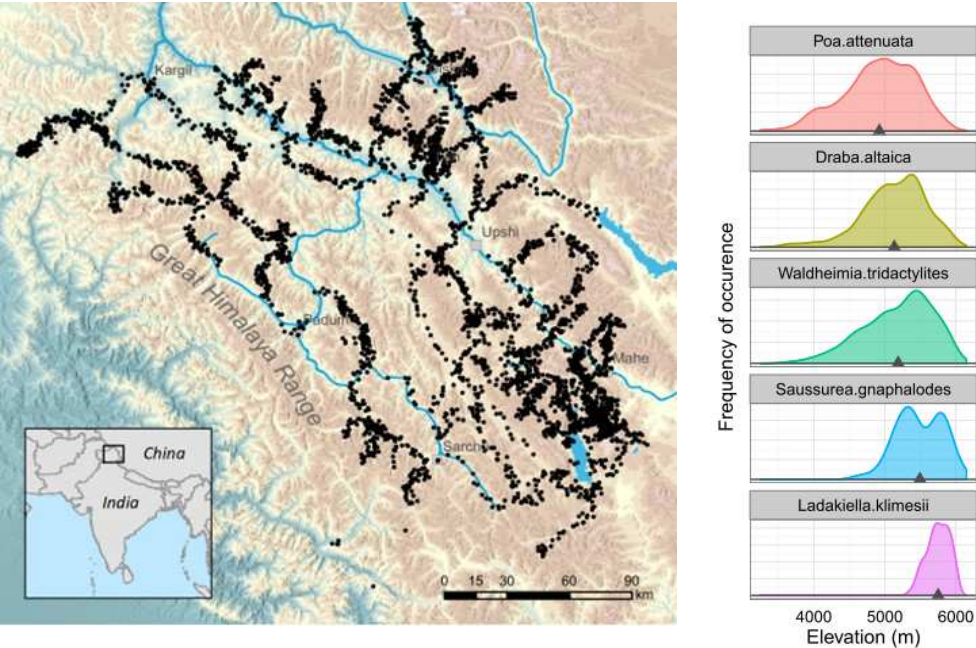
JAN:	$y = -2.9535x + 5962$	0.296	0.343
FEB:	$y = -0.5429x + 1116.8$	0.035	0.722
MAR:	$y = 0.1714x - 316.16$	0.006	0.882
APR:	$y = -2.8857x + 5827.8$	0.286	0.274
MAY:	$y = -2.7714x + 5591.9$	0.185	0.394
JUN:	$y = -4.6x + 9266.7$	0.734	0.029
JUL:	$y = 1.1714x - 2348.8$	0.185	0.394
AUG:	$y = -0.1786x + 369.68$	0.005	0.887
SEP:	$y = -2.4643x + 4965.8$	0.763	0.010
OCT:	$y = -3.6286x + 7309.1$	0.312	0.249
NOV:	$y = -4.7674x + 9598.4$	0.663	0.094
DEC:	$y = -2.2674x + 4574.5$	0.250	0.391

Supplementary Table S2. List of species recorded in 80 permanent plots 1 x 1 m. New species found during the second survey are in bold. GLMM model, with plot identity as a random effect factor and year as fixed effect factor, was used to test for species-specific changes in cover abundance between the two sampling periods (all *P* values <0.1 indicate species cover decrease). Also shown are plant heights and elevational minima, maxima, optima and ranges.

Species	P	Plant height	Elevational distribution (m)			
			Min	Mean	Max	Range
		(cm)				
<i>Actinocarya acaulis</i>	ns	2.0	4615	5295	5625	1010
<i>Aphragmus oxycarpus</i>	0.025	4.3	3420	5334	6000	2580
<i>Arenaria bryophylla</i>	0.033	3.5	4105	5397	5910	1805
<i>Artemisia minor</i>	ns	11.0	4362	5069	5700	1339
<i>Aster flaccidus</i>	ns	10.0	3500	4667	5675	2175
<i>Astragalus confertus</i>	ns	13.0	4410	5422	5795	1395
<i>Astragalus hendersonii</i>	ns	5.9	4924	5342	5595	671
<i>Astragalus strictus</i>	ns	15.0	3145	4489	5525	2380
<i>Carex borii</i>	ns	5.8	4620	5306	5730	1110
<i>Carex sagaensis</i>	0.000	8.1	3600	5149	5800	2205
<i>Carex pseudofoetida</i>		12.0	3130	4855	5875	2745
<i>Delphinium brunonianum</i>	ns	31.6	3680	5079	5700	2020
<i>Desideria pumila</i>	0.000	2.0	5191	5813	5990	799
<i>Draba altaica</i>	0.000	2.5	3500	5409	6150	2500
<i>Draba lasiophylla</i>	ns	8.4	3330	5028	5725	2395
<i>Draba oreades</i>	0.032	1.9	4250	5616	6010	1760
<i>Elymus schrenkianus</i>	ns	35.0	3331	4536	5582	2251
<i>Eritrichium hemisphaericum</i>	0.000	1.7	5225	5942	5990	765
<i>Festuca non-coelestis</i>	ns	11.0	4775	5412	5738	963
<i>Halerpestes sarmentosa</i>		4.2	2680	2824	5485	2805
<i>Hedinia tibetica</i>		12.0	4575	5434	5815	1240
<i>Kobresia pygmaea</i>	ns	3.5	4000	5171	5766	1766
<i>Kobresia schoenoides</i>	ns	20.3	2680	4602	5650	2970
<i>Koenigia islandica</i>		10.0	2880	5007	5725	2845
<i>Ladakiella klimesii</i>	0.000	4.5	5350	5862	6150	660
<i>Nepeta longibracteata</i>	ns	4.2	4390	5316	5775	1485
<i>Oxytropis chiliophylla</i>	0.106	8.4	4640	5266	5795	1165
<i>Oxytropis platysema</i>	0.000	9.5	4260	5228	5795	1545
<i>Pegaeophyton scapiflorum</i>	0.014	2.8	4020	5567	5920	1900

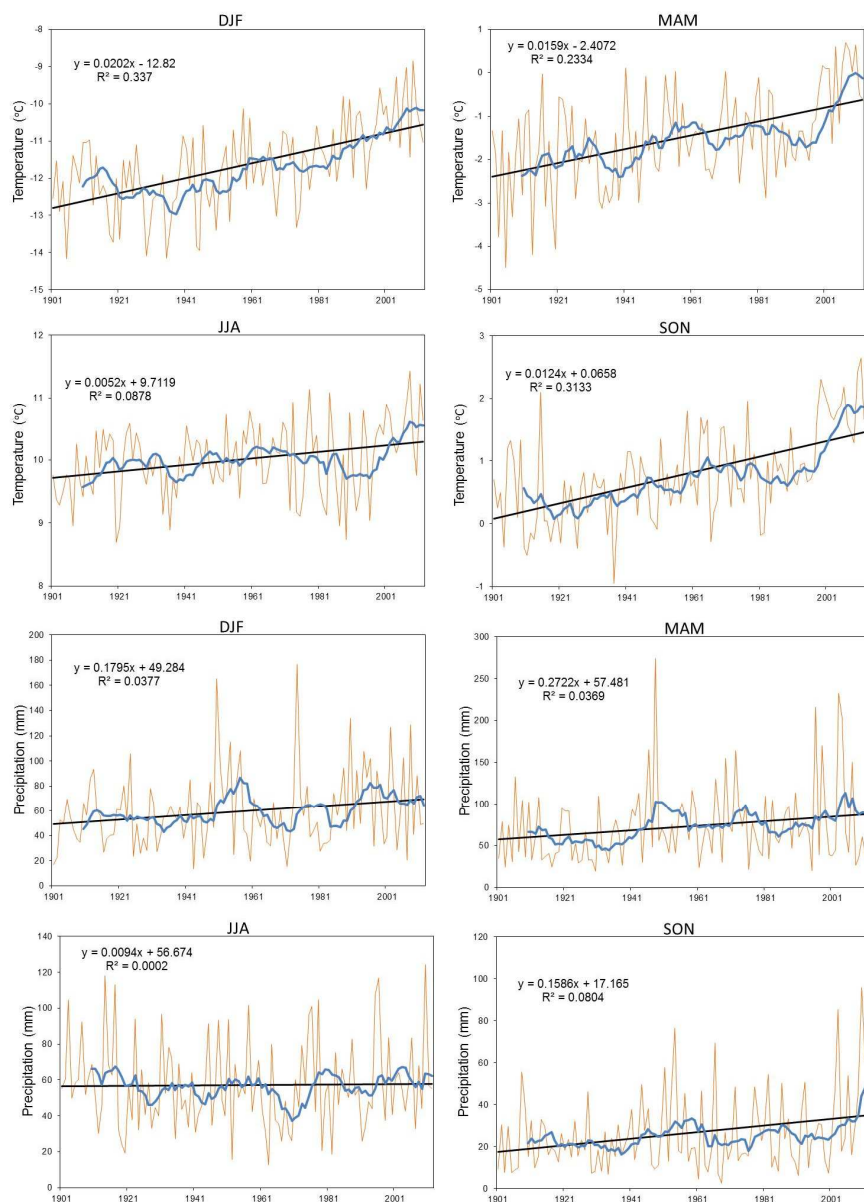
HIGH-ELEVATION VEGETATION DYNAMICS AND CLIMATE CHANGE

<i>Poa attenuata</i>	0.000	35.0	3245	5007	6150	2755
<i>Potentilla gelida</i>		13.1	3325	4967	5625	2300
<i>Potentilla pamirica</i>	0.000	4.3	3839	5157	5885	2046
<i>Primula macrophylla</i>	ns	17.7	3965	5228	5785	1820
<i>Saussurea bracteata</i>	ns	3.4	4540	5213	5740	1200
<i>Saussurea glacialis</i>	0.005	4.5	4250	5542	6010	1760
<i>Saussurea inversa</i>	0.019	3.7	4660	5843	6150	1310
<i>Saussurea hypsipeta</i>	0.035	7.0	4870	5853	6060	1190
<i>Saxifraga cernua</i>	0.064	8.5	4350	5462	5885	1535
<i>Saxifraga nanella</i>	0.006	1.7	4970	5765	5995	1025
<i>Stellaria brachypetala</i>	ns	20.0	2898	4103	5420	2522
<i>Stellaria decumbens</i>	0.000	2.5	3410	5630	6010	2600
<i>Stellaria depressa</i>	0.057	17.5	3660	5101	5950	2290
<i>Stipa subsessiliflora</i>	ns	31.0	4038	4944	5690	1653
<i>Thalictrum alpinum</i>	ns	11.1	3745	4956	5675	1930
<i>Thylacospermum caespitosum</i>	0.023	30.0	4200	5461	5960	1760
<i>Waldheimia tridactylites</i>	ns	7.0	3615	5424	6150	2415



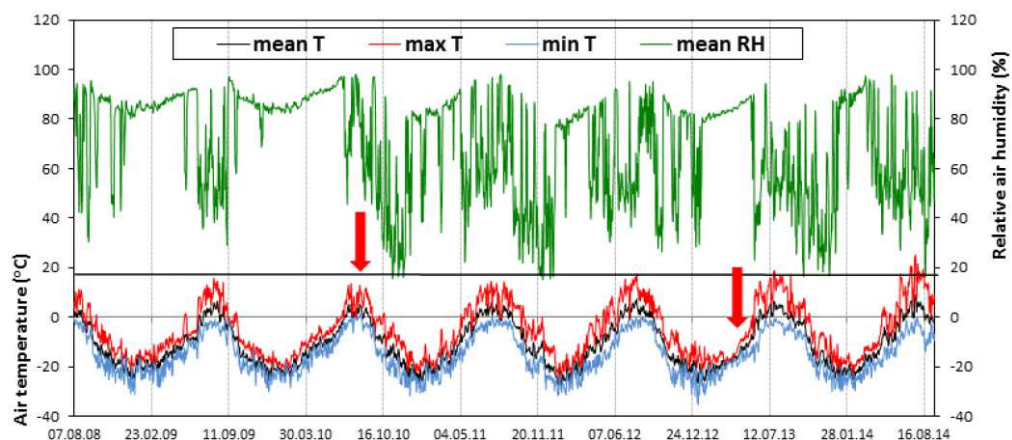
Supplementary Figure S1. Study area in Ladakh, Indian NW Himalayas. Floristic data were collected at 4,150 localities (black dots) spread over Ladakh area (to facilitate the visibility of overlapping plots, we randomly shifted plot coordinates up to 2 km). The map was created in software ArcGIS, ver. 10.1 (www.esri.com). The background relief was derived from the SRTM data (<http://www2.jpl.nasa.gov/srtm/>, the version provided by <http://srtm.csi.cgiar.org>), and the river network was adapted from OpenStreetMap (www.openstreetmap.org).

Elevational ranges of five vascular plant species that significantly moved upwards in the past ten years in Eastern Ladakh near Tso Moriri Lake, where repeated monitoring of alpine and subnival vegetation was conducted. The curves represent the density distribution of sites with individual species presence and black triangles represent the mean elevation of these sites.

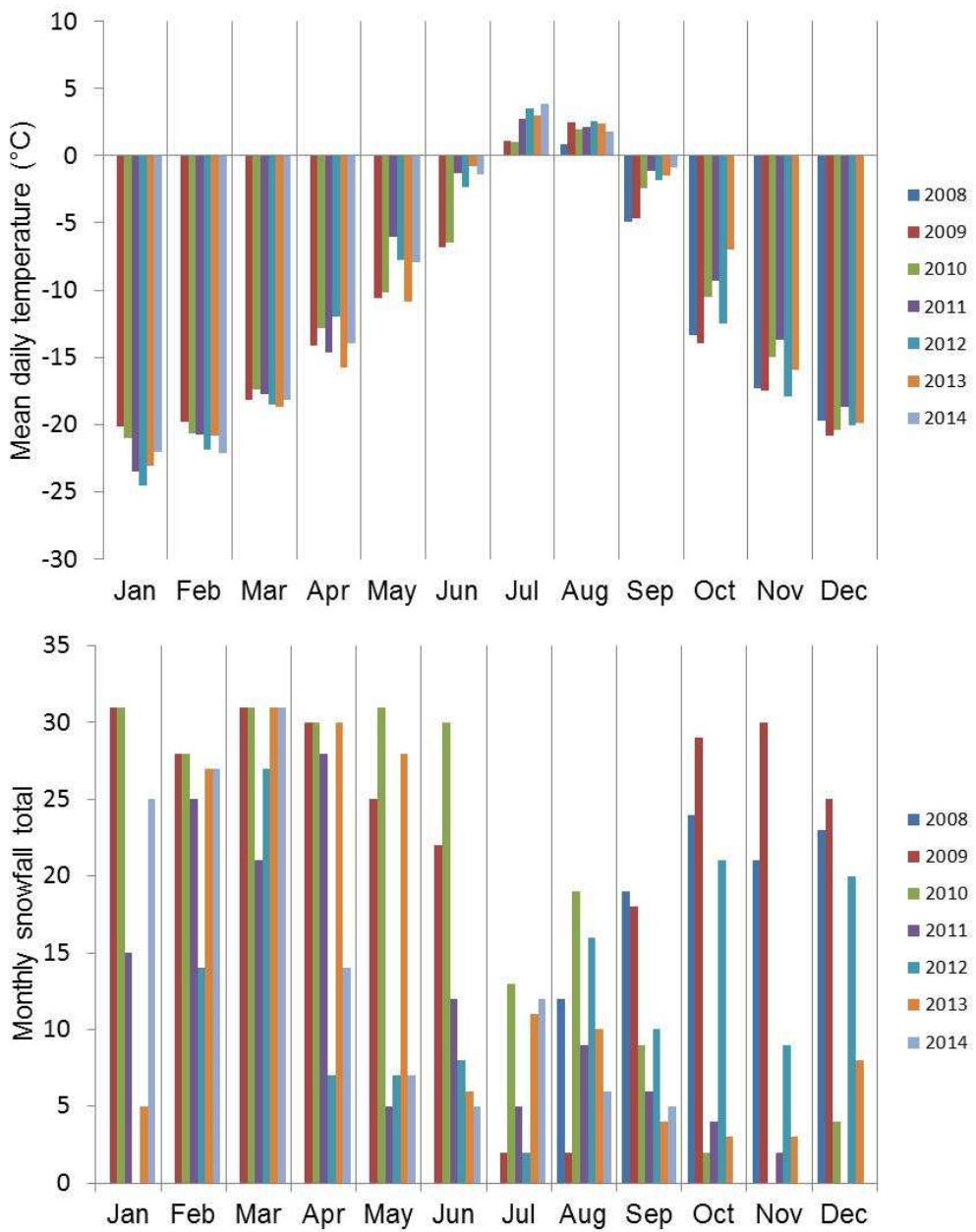


Supplementary Figure S2. The long-term climatic changes in Ladakh. The average temperature and precipitatin in the study area for the spring (MAM), summer (JJA), autumn (SON) and winter (DJF) periods, with smoothed values (10 year running mean) and fitted linear regression. The $0.5^\circ \times 0.5^\circ$ gridded monthly climatic data from CRU TS 3.22 (Harris 2014) were used (<http://climexp.knmi.nl>). Data cover the period from 1901 – 2014.

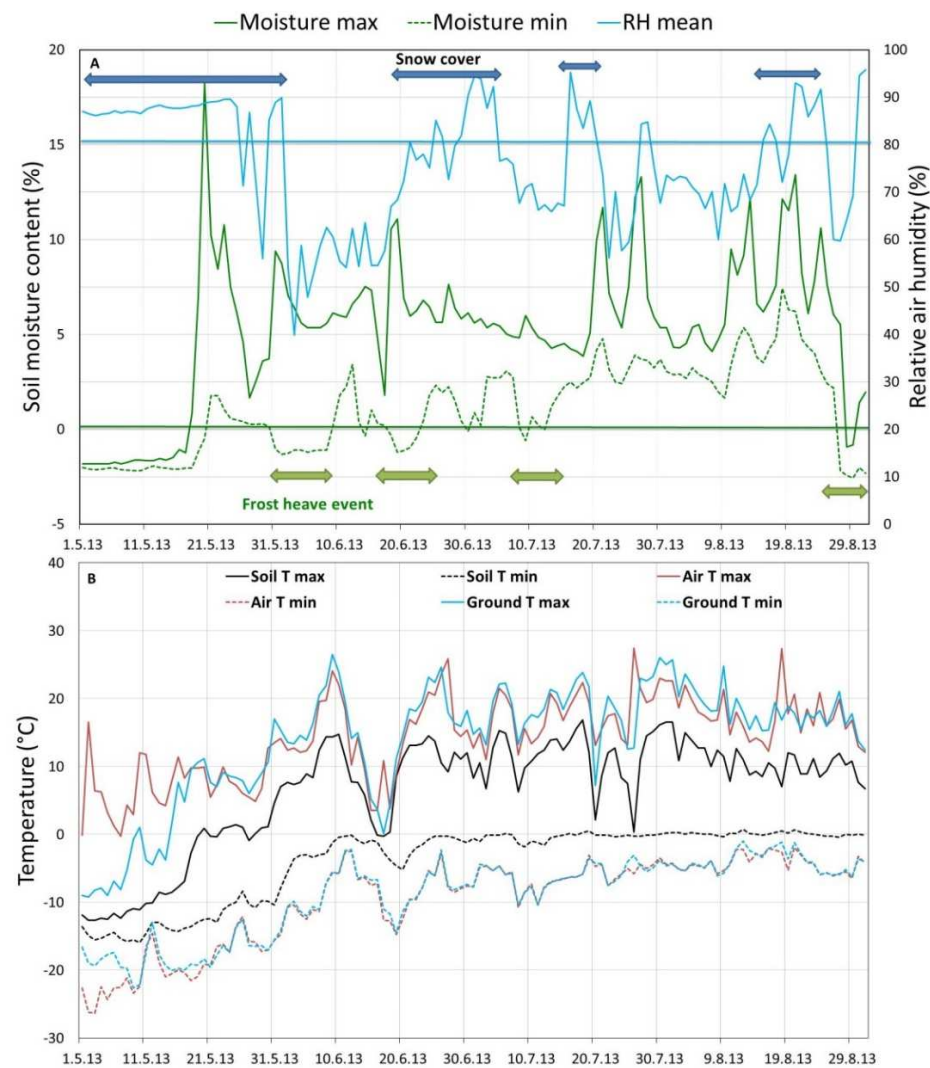
Harris I, Jones PD, Osborn TJ, Lister DH (2014) Updated highresolution grids of monthly climatic observations—the CRU TS3.10 dataset. *Int J Climatol* 34:623–642.



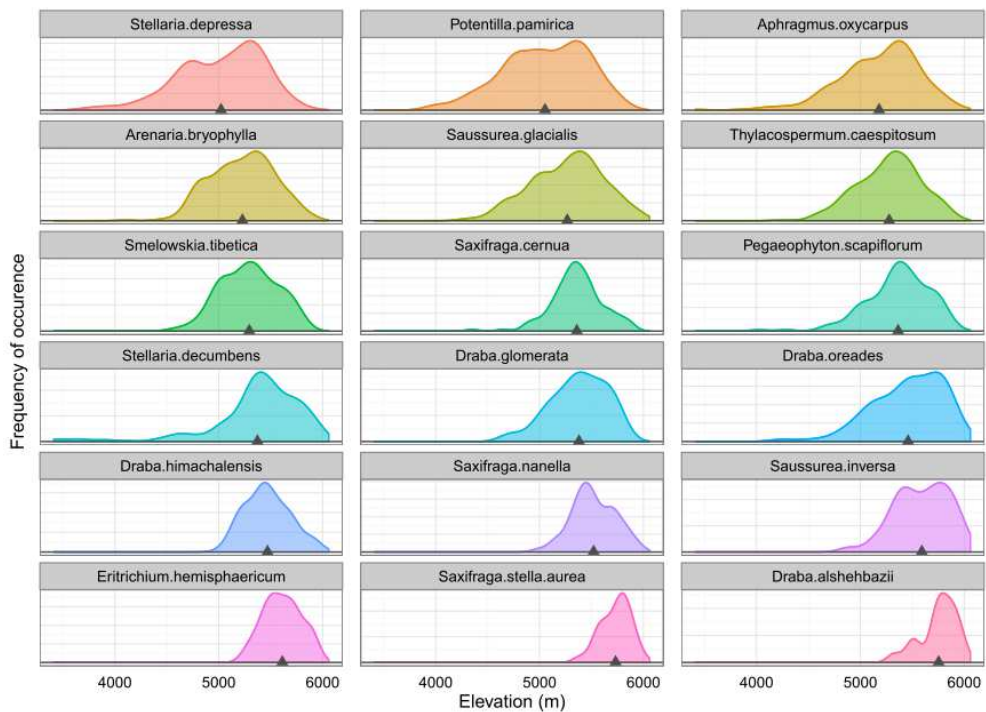
Supplementary Figure S3. Climate at 5900 m. The seven-year-long records of air temperature (T) and relative air humidity (RH), measured hourly 3 cm above the ground from August 2008 to September 2014, by a HOBO™ U23 Pro v2 logger (www.onsetcomp.com).



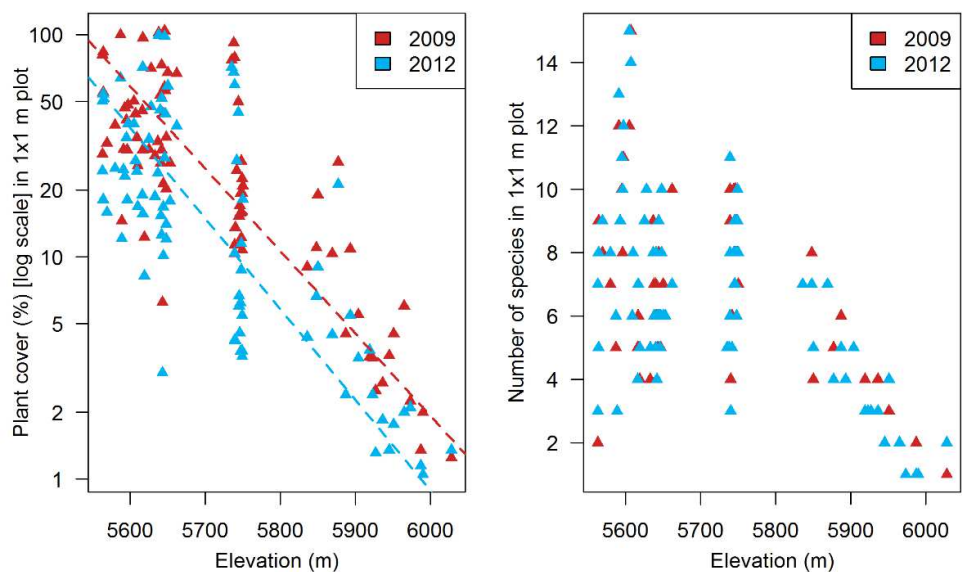
Supplementary Figure S4. Climate at 5900 m. Mean daily temperature and snowfall totals for individual months from August 2008 to September 2014 (see Table S2 for significant temporal trends).



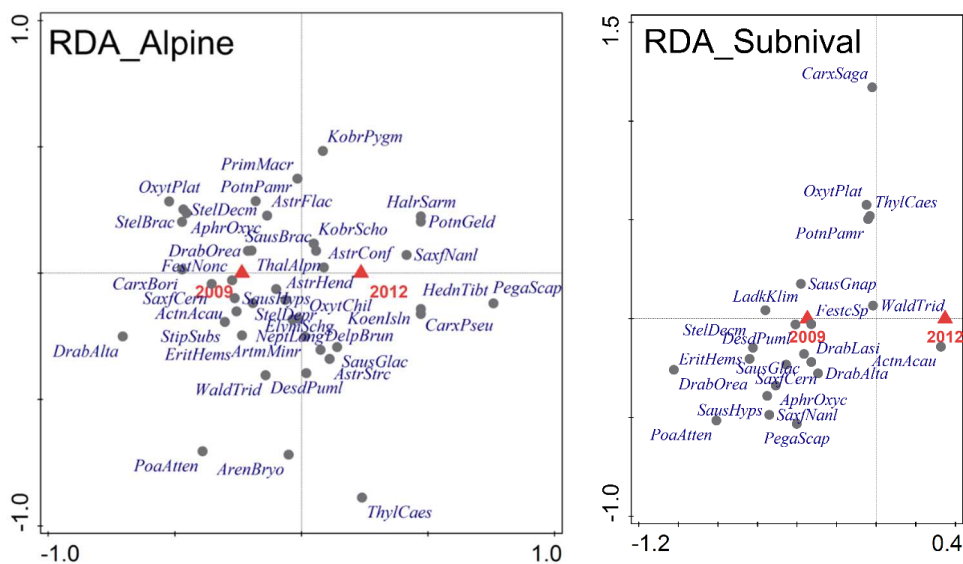
Supplementary Figure S5. Example of frost heave events at 5900 m. Continuous snow cover in 2013 lasted from February until May and was unusually deep, followed by a warm June and July. This increased the risk of frost heaving (soil water freezes and melts the same day) as an upwards swelling of soil during night freezing caused by an increasing presence of ice in wet soil after snowmelt. Days with snow cover/snowfall (blue arrows) were derived from relative air humidity (RH) measurements (mean daily values above 80%) verified by automatic PlantCam™ camera snapshots. Snow temperature T (-8 cm) and soil moisture (0 to -10 cm), ground T and air T (+10 cm) were measured by TOMST logger.



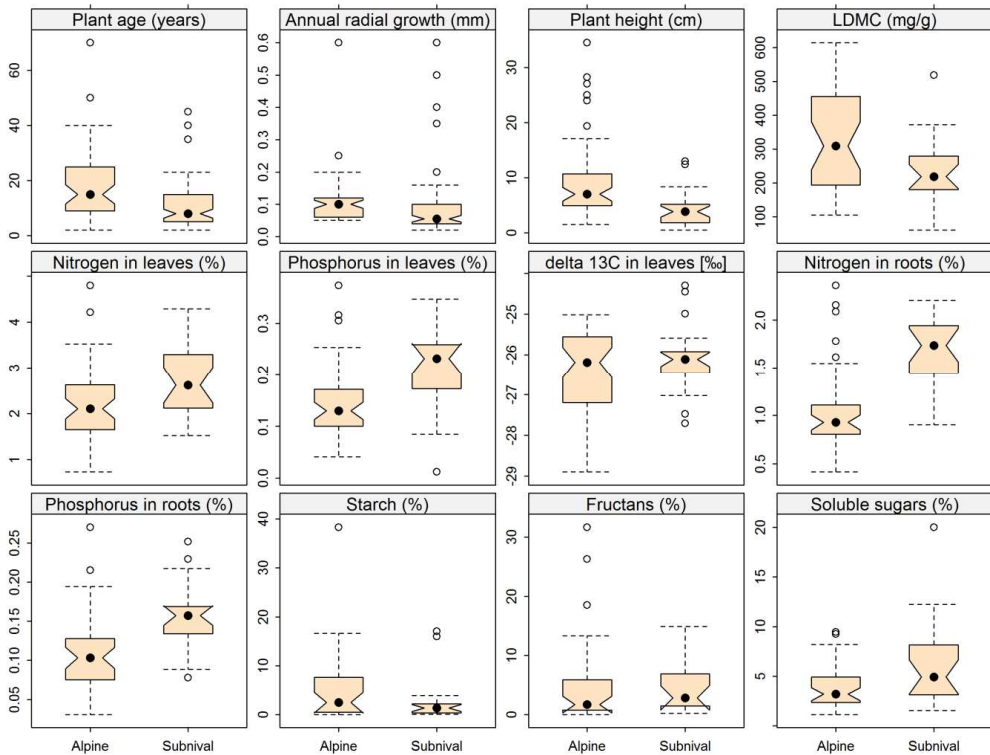
Supplementary Figure S6. Species distribution along elevation gradient in Ladakh. Only species found above 5800 m are shown. The curve represents the density distribution of sites with individual species presence and the black triangle represents the mean elevation of these sites. The subnival vegetation of Ladakh is not dominated by high-elevation specialists, but rather a mixture of species with heterogeneous distribution patterns. The prevailing are species centered at much lower elevations and having wide vertical ranges.



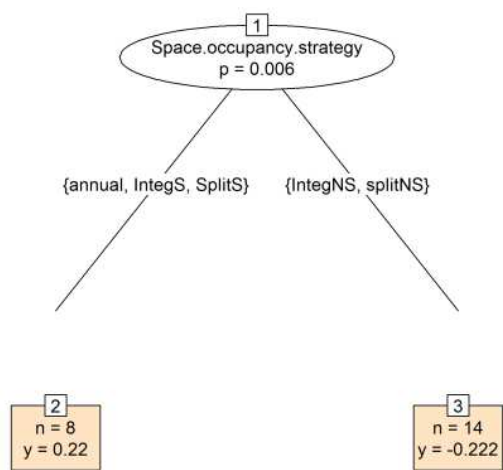
Supplementary Figure S7. Changes in vegetation cover and number of species of subnival vegetation between 2009 and 2013 in 1 x 1 m plots.



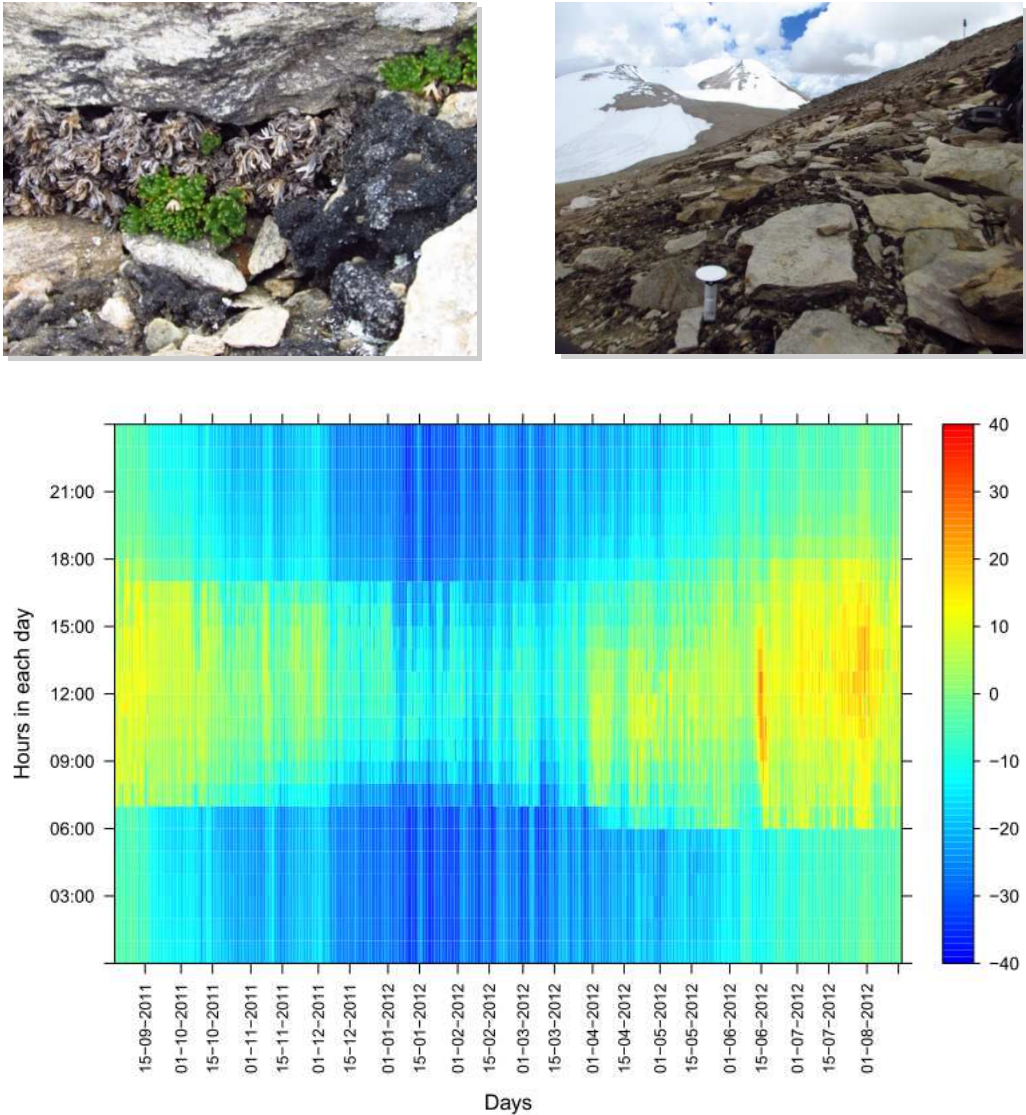
Supplementary Figure S8. RDA ordination diagrams for compositional changes in alpine and subnival vegetation between 2009 and 2012. The first four letters of generic and species names are given (see Supplementary Table S2 for full names).



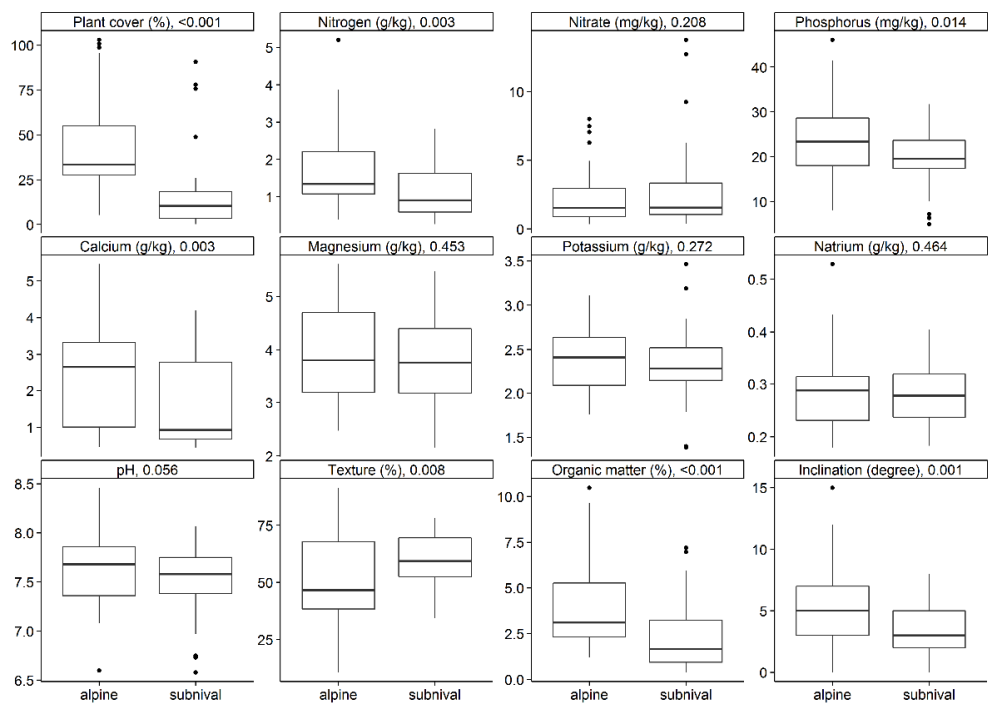
Supplementary Figure S9. Comparison of plant life history traits between alpine (43 species) and subnival species (24 taxa above 5800 m) based on their mean values. Soluble sugars include raffinose family oligosaccharides, sugar alcohols (glycerol, xylitol, arabitol, dulcitol, adonitol, myoinositol sorbitol, mannitol) and simple sugars (galactose, glucose, fructose, sucrose, D-galactosamine, rhamnose, arabinose, D-Glucosamine, xylose, mannose, D-allose, ribose, melibiose, glucoheptose, lactose, D-rafinose, D-talose, cellobiose, maltose), quantified using high-performance anion exchange chromatography with pulsed amperometric detection (for details see Chlumská et al. 2014). Boxes represent 25% – 75% of values, dots near the middle of the box are the medians, whiskers indicate the 1.5 interquartile range, and dots are outliers. Notches in the boxes indicate the significance of between-group differences: if notches of two groups do not overlap this is evidence that medians of these groups differ significantly. Notches that extend above the 75th percentile or below the 25th percentile indicate the high inter-sample variance and possible invalidity of the test that is based on the assumption of asymptotic normality of the median.



Supplementary Figure S10. Life-history traits and ecological indicator values predicting species abundance changes in the subnival vegetation using a conditional inference tree.



Supplementary Figure S11. The highest and coldest site in the study area (6150 m) with vascular plants. (Upper right) *Waldheimia tridactylites* population in the leeward side of a large boulder. The photograph was taken by the first author (J.D.) in August 2012. (Upper left) TOMST® TMS station recording air, surface and soil temperatures and soil moisture contents at 15 min intervals. Chamser Kangri peak (6660 m a.s.l.) in the background. (Bottom) Diurnal and seasonal course of air temperatures.



Supplementary Figure S12. Plot-level comparison of soil chemismums, plant cover and slope. Comparison of concentrations of total plant cover, soil nutrients, soil reaction (pH), soil texture (fraction of particles bigger than 0.5 mm in diameter), and slope inclination of permanent monitoring plots in the alpine and subnival vegetation on the western slope of Chamser Kangri, Tibetan Plateau. Generalized linear models were used to test for statistical differences between habitats (type I error estimate is given behind variable names).

CHAPTER II

Gardening in the zone of death: an experimental assessment of the absolute elevation limit of vascular plants

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Photo on previous page: our team establishing a transplant experiment under the Chamser Kangri Peak at 6100 m a.s.l., Rupshu region, eastern Ladakh.

Gardening in the zone of death: an experimental assessment of the absolute elevation limit of vascular plants

Abstract

Vascular plants in the western Tibetan Plateau reach 6000 m-the highest elevation on Earth. Due to the significant warming of the region, plant ranges are expected to shift upwards. However, factors governing maximum elevational limits of plant are unclear. To experimentally assess these factors, we transplanted 12 species from 5750 m to 5900 m (upper edge of vegetation) and 6100 m (beyond range) and monitored their survival for six years. In the first three years (2009–2012), there were plants surviving beyond the regional upper limit of vegetation. This supports the hypothesis of dispersal and/or recruitment limitation. Substantial warming, recorded in-situ during this period, very likely facilitated the survival. The survival was ecologically a non-random process, species better adapted to repeated soil freezing and thawing survived significantly better. No species have survived at 6100 m since 2013, probably due to the extreme snowfall in 2013. In conclusion, apart from the minimum heat requirements, our results show that episodic climatic events are decisive determinants of upper elevational limits of vascular plants.

Introduction

Distribution of many species in alpine regions has been documented to be shifting as a result of the ongoing climate change^{1–5}. Alterations of warming and precipitation regimes affect the performance of species particularly at their range margins. Alpine plants are particularly sensitive to climate change so high mountains provide suitable natural laboratories for tracing climate-induced biological responses⁶. In alpine regions, climate change may ameliorate growing conditions for plants and, thus, may open unpopulated habitats. The pace of colonisation will depend on the dispersal capacities of the upper populations of species. When climate change leads to the deterioration of conditions, alpine perennial plants may still persist as they are capable of surviving in apparently suboptimal conditions for considerable periods of time, even a few decades⁷. The net effect of climate change is, however, more complex and likely combines an amelioration of some aspects of the environmental conditions and the deterioration of others. For example, a period of warmer summers may support generative reproduction and result in establishment of populations at higher elevations, while longer and more snowy winters may eliminate them, especially at their uppermost ecophysiological limits⁸.

The highest elevation limit of continuous, although sparsely scattered, vegetation in the world has been reported from the westernmost part of the Tibetan Plateau, and it is located at ca. 6000 m^{8,9}. This part of the Transhimalaya, i.e. the arid land north of the main Himalaya Range, provides the most suitable conditions for plant occurrence at extreme altitudes¹⁰. The factors facilitating its habitability include (1) favourable geomorphology (gentle slopes of the plateau preventing serious erosion and substrate instability), (2) arid climate (< 100 mm yr⁻¹; preventing extensive glaciation; the snow line located hundreds of meters above the vegetation limit; limited frost-heave and solifluction in dry substrates), (3) suitable geology such as metamorphic bedrock producing relatively fine substrates at a limited occurrence of coarse screes, and (4) location within a subtropical belt (high solar input). All of these conditions

predominate over the whole south and west Tibetan Plateau (which stretches to East Ladakh), where even higher limits of continuous vegetation can be expected. To the best of our knowledge, such higher limits have not been reported so far. The highest record was reported from the south of the Tibetan Plateau, from the region north of Mt. Everest¹¹ which already lies in the rain shadow of the main Himalaya Range. This record of *Saussurea gnaphalodes* at 6400 m originates from the 1930s and has never been confirmed since; apart from the doubts about the correctness of the elevation measurements and the absence of microclimatological characterisation of the site, records like this tell little about the elevation limits of continuous vegetation on Earth.

Climate change in Ladakh, as well as the rest of the western Himalaya and the adjacent part of the Tibetan Plateau, includes undoubted rapid warming¹²⁻¹⁴. The rate of warming may be different at higher elevations¹⁵, e.g. in Tibetan Plateau it does not continue to rise above 4800 m and remains at a high level with a slight decline at the highest elevations¹⁶. Warming leads to an extension of the growing season due to earlier snowmelt and later snowcover, and helps to expose colonizable substrates, e.g. after glacier retreat¹⁷. These phenomena enable vegetation to expand beyond its range. Precipitation change in the region exhibits a more local pattern and a study explicitly concerning our study region is not known to us. In the surrounding area, where studies have been made, there is mainly an indication of increased precipitation^{13,14,18-22}, although reports of a decrease have also been made²³. The increasing trend of summer and winter precipitation over the Himalaya is associated with an increasing trend in precipitation extremes connected to the changing pattern of the summer monsoons^{20,24-27}, and an enhancement in the strength and frequency of winter westerly disturbances^{18,28-31} (extratropical storms driven by the Westerlies, that bring sudden winter rain and snow to the northwestern parts of the Indian subcontinent), respectively. Indeed, over the last decade, Ladakh experienced unusual summer storms, causing massive flash floods³². In addition, years 2013 and 2015 have had the most extreme snowfall in the last 50 years.

In the region of study, the subnival zone of Eastern Ladakh, snowfall occurs all year round. An increase in precipitation may cause thicker snow cover which potentially leads to a delayed disappearance of snow, shortening of the vegetation season, enlargement of the permanent snowfields and the eventual concealment of habitable places. Wetter substrates are also more susceptible to frost heave, a serious factor complicating seedling establishment and even the persistence of mature plants⁶ adapted to the arid substrates of cold, high-altitude deserts. Thus, the factors associated with higher water input into the ecosystem can make the survival at the extreme elevations harder³³ and, in the long term, may contribute to the shrinkage of the subnival zone.

As the effect of increased precipitation on the direction of the vegetation shift may be opposite to the effect of warming, the principal questions are whether the current upper species ranges are (1) in an equilibrium state with the current climate, (2) lagging behind the climate amelioration (warming) due to dispersal limitations, i.e. there are uninhabited habitats available beyond the current range, or (3) paying the extinction debt (mortality time lag) because of the deterioration of conditions (prevailing adverse effect of higher water input or more extreme variability of cold and warm conditions). A flawless approach to these hypotheses would be based on a sowing experiment³⁴. However, severe conditions on the edge of vegetation does not allow every-year generative reproduction or seedling establishment. In fact, such propitious seasons are rather rare and the populations at the upper range margins are often demographic sinks³⁵, i.e. established and maintained thanks to seeds dispersed from lower altitudes^{6,36}. Therefore, if the experimental seeds were sown with no success, it could just mean that we failed to match the rarely occurring suitable year. Thus, we adopted an alternative approach, and conducted a transplant experiment to test our three hypotheses of species response. The survival/persistence of mature plants transplanted beyond their current range would suggest dispersal and/or recruitment limitation, and, accordingly, the hypothesis of habitat limitation would be rejected. If no plants survived, habitat limitation would be concluded to be the primary cause.

Growth in cold environments is primarily determined by temperature⁶, stressing the importance of sink-limitation; even if there are sufficient resources, the prevailing low temperatures slow their incorporation into new tissues as enzymatic systems run in suboptimal conditions. Consequently, alpine plants often have higher concentrations of nutrients in their tissues³⁷. In contrast, there is some indication that at extreme elevations the pattern changes and low nutrient uptake can limit growth³⁸; nutrient availability may play a greater role than previously thought. Fertilization was therefore included in the experiment to examine possible effects of nutrient limitation.

The main objectives of this study were to examine if (1) mature plants transplanted beyond their uppermost range margins are able to survive, (2) microclimate amelioration and nutrient addition support survival, and (3) particular functional traits are associated with better survival.

Results

Microclimate

Growing season length at the transplant sites decreased with elevation, and its year-to-year variation was considerable (Table 1). Temperature measurements conducted during August 2008–August 2014 at 5900 m showed significant increase in mean temperature of summer months, namely June (+1.23 °C per year), July (+0.59 °C per year), and September (+0.69 °C per year) (Fig. 1; Supplementary Table S1). The same trend was recorded for mean daily maximum temperature. No trends were recorded for mean temperature of winter months, except for a cooling in February (−0.38 °C per year).

Survival of species

Table 1. Microclimatic characteristics of sites at contrasting elevations in Changthang, eastern Ladakh. Temperature was recorded by TMS loggers 10 cm deep in the soil, on the surface, and 10 cm above the surface during 2009–2015 at hourly intervals. Klimeš and Doležal⁸ recorded temperature 5 cm deep in the soil and 10 cm above the surface during 2002–2003 at hourly intervals. Growing season is taken as a period with daily mean temperature of soil above 0 °C. Growing degree-days (above 0 °C) were calculated from hourly temperature recordings. *indicate sites where vascular plants naturally occurred, **values for the year 2014 (Source, Edge, Beyond-range, Outpost), and for the year 2003 (Klimeš & Doležal⁸).

At the source site at 5750 m, all but four species (*Desideria pumila*, *Ladakiella klimesii*, *Stellaria decumbens*, *Waldheimia tridactylites*) survived across the treatments but with variable success (Table 2). At the edge site at 5900 m, with the exception of *Saussurea inversa* all species survived, but again the numbers of surviving individuals differed a lot. *Saxifraga cernua* showed the second highest survival rate here, with six flowering individuals. This is especially

Site	Source	Edge	Beyond-range	Outpost	Klimeš & Doležal ⁸					
Treatment	Control	Enclosure	Enclosure		6030*		6150*		6160	
Elevation [m a.s.l.]	5750*	5900*	6100	6150*	West		Southwest		West	
Exposure	West		Northwest		Northwest		Southwest		West	
Temperature [°C]**	Soil	Surface	Soil	Surface	Soil	Surface	Soil	Surface	Soil	Air
Annual mean	-6.9	-8.2	-8.7	-9.6	-11.1	-10.3	-10.8	-12.2	-8.1	-11.6
Absolute maximum	16.1	23.3	12.9	22.8	22.5	9.8	15.4	20.3	12.0	23.8
Absolute minimum	-22.3	-29.9	-22.5	-28.4	-38.9	-26.6	-29.1	-40.6	-24.3	-31.0
Maximum daily range	13.8	27.0	12.8	26.1	29.7	11.1	18.3	26.9	13.6	30.1
Year	2003	2003	2003	2003	2003	2003	2003	2003	2003	2003
Growing season length [days] / Degree days >0 °C	92/251	70/268	55/95	47/121	111/241	89/163	19/19	19/19	11.1	21.4

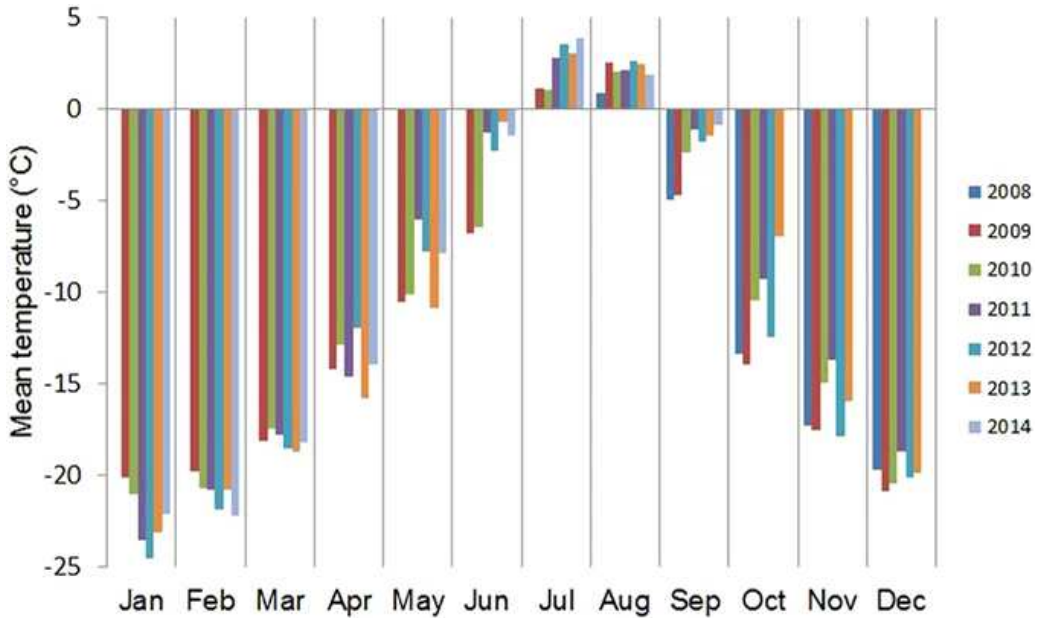


Figure 1. Mean monthly air temperature of Chamser Kangri Massif for period 2009–2014. Recorded 30 cm above ground ca. 1 km from experimental sites at 5900 m a.s.l. in hourly intervals.

noteworthy, as the 5900 m site lay 60 metres above the species' natural limit. At the beyond-range site at 6100 m, individuals of three species survived (*Saxifraga cernua*, *S. nanella* and *Poa attenuata*). Seven surviving individuals of these species were inside the enclosure, but there were also two individuals in unsheltered plots (both control and fertilized). These three species had the highest survival rates at all the sites. Since 2013, no individuals were surviving at the beyond-range site.

None of the surviving plants at 6100 m was bearing flowers in 2012. At 5900 m, 18 out of 51 surviving plants were flowering while only two individuals out of 43 (both *P. attenuata*) were flowering at 5750 m (Table 2). Growth analysis was mostly inconclusive due to low numbers of surviving individuals (Supplementary Table S2). However, plants at 6100 m generally decreased in size and height and had lower number of shoots in 2012 compared to 2010, while at 5900 m we recorded size gains and increasing numbers of shoots.

Table 2. Transplanted species. Fam-family (A-Asteraceae, B-Brassicaceae, C-Caryophyllaceae, P-Poaceae, S-Saxifragaceae), distribution in East Ladakh (Min alt-low elevation limit, Max alt-upper elevation limit; *indicate species found at a remote outpost beyond the range of continuous vegetation), number of surviving individuals in 2012 in respective treatment plots (maximum number is 8; number in brackets indicate number of individuals flowering in 2012, otherwise there were no flowering individuals; C-control plot, F-fertilised plot, E-enclosure, EF-enclosure with fertilisation, Σ -sum of surviving species in all plots).

Species surviving at upper elevation had epigeogenous rhizome ($p = 0.0002$, $p_{corr} = 0.005$, $R^2_{adj} = 0.77$), lower $\delta^{13}C$ ($p = 0.008$, $p_{corr} = 0.18$, $R^2_{adj} = 0.51$), higher C:N ratio ($p = 0.02$, $p_{corr} = 0.446$, $R^2_{adj} = 0.41$) and lower LPC ($p = 0.0002$, $p_{corr} = 0.961$, $R^2_{adj} = 0.31$).

Effect of treatments

Site elevation affected survival ($\chi^2 = 35.310$ on 2 d.f., $p < 0.001$; Fig. 2). Survival at the edge site was not significantly different from that at the source site (OR = 0.9, $z = -0.177$, $p = 0.859$), while survival at the beyond-range site was considerably lower (OR = 0.04, $z = -5.224$,

Species	Fam	Min alt	Max alt	Source site (5800 m)						Edge-site (5950 m)						Beyond-range site (6100 m)					
				C	F	E	EF	Σ	C	F	E	EF	Σ	C	F	E	EF	Σ			
<i>Desideria pumila</i> (Kurz) Al-Shehbaz	B	5380	5990	—	—	—	—	0	—	—	3	—	—	—	—	—	—	0			
	B	4800	6010	—	—	4	1	5	2	—	—	2	—	—	—	—	—	0			
<i>Ladakiella klimesii</i> (Al-Shehbaz) D.A. German & Al-Shehbaz	B	5350	6150*	—	—	—	—	0	—	—	1	—	—	—	—	—	—	0			
<i>Poa attenuata</i> Trin.	P	4540	6000	—	1	3(1)	1(1)	5	5(1)	1(1)	8(5)	3(3)	17	—	—	1	—	1			
<i>Saussurea glacialis</i> Herder	A	4595	6150*	2	1	1	—	4	—	—	1	—	1	—	—	—	—	0			
<i>Saussurea inversa</i> Raab-Straube	A	5250	6150*	3	—	1	—	4	—	—	—	—	0	—	—	—	—	0			
<i>Saxifraga cernua</i> L.	S	4650	5890	7	1	8	—	16	2	—	7(3)	3(3)	12	1	1	4	—	6			
<i>Saxifraga nanella</i> Engl. & Irmsch.	S	5150	5995	2	—	5	—	7	2	—	6(2)	—	8	—	—	2	—	2			
<i>Stellaria decumbens</i> Edgew.	C	4560	6060	—	—	—	—	0	1	—	2	—	3	—	—	—	—	0			
<i>Thylacospermum caespitosum</i> (Cambess.) Schischk.	C	4550	5960	1	—	1	—	2	2	—	—	—	2	—	—	—	—	0			
<i>Waldheimia tridactylites</i> Kar. & Kir.	A	4820	6150*	—	—	—	—	0	2	—	—	—	2	—	—	—	—	0			
			total	15	3	20	1	43	11	0	7	0	51	1	1	7	0	9			

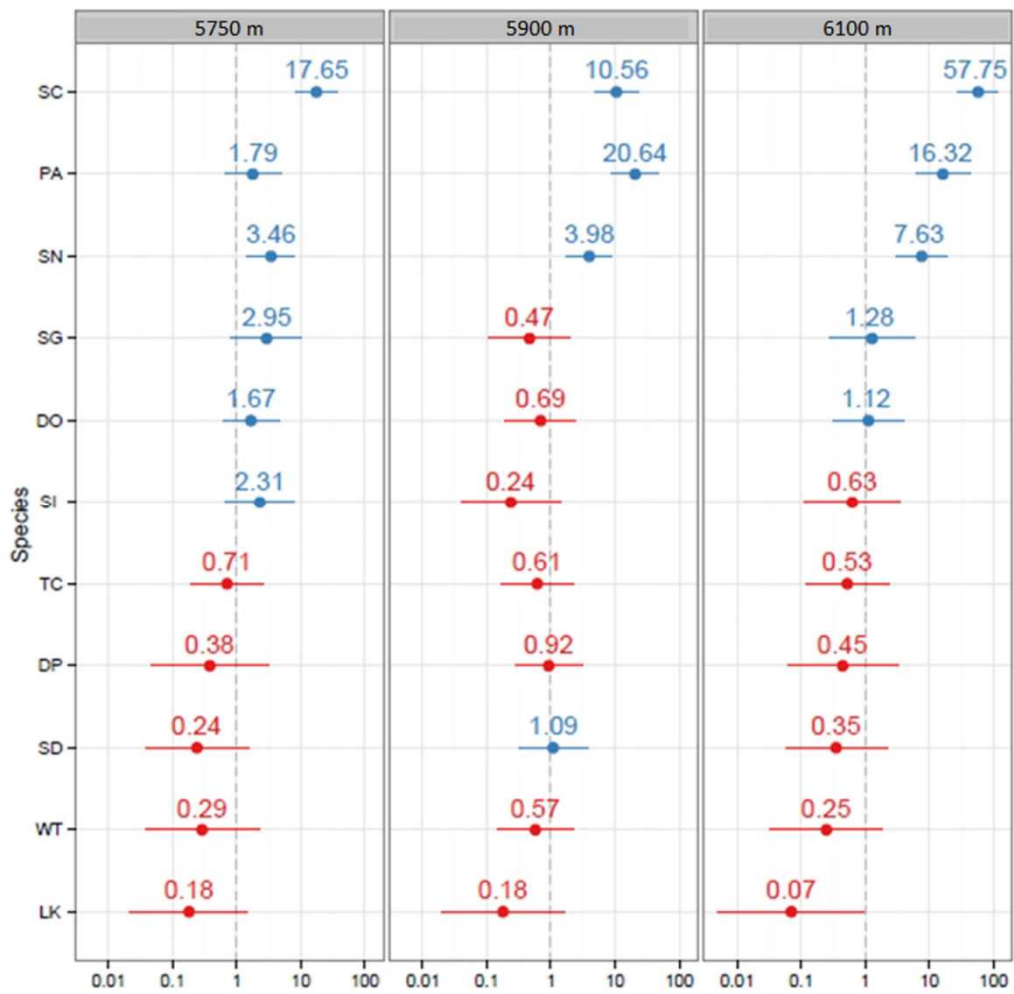


Figure 2. Survival of species-odds-ratios for random effect at species level for each elevation. Survival higher than expected at all elevations was consistently found for *Saxifraga cernua* (SC), *Poa attenuata* (PA) and *Saxifraga nanella* (SN). Low survival rate of *Ladakiella klimesii* (LK) was evident in all treatments. Other species showed an intermediate or inconsistent response to experimental transplantation along the elevation gradient (DO–*Draba oreades*, DP–*Desideria pumila*, SD–*Stellaria decumbens*, SG–*Saussurea glacialis*, SI–*Saussurea inversa*, TC–*Thylacospermum caespitosum*, WT–*Waldheimia tridactylites*).

$p < 0.001$). Overall, stone enclosures had a positive effect on plant survival (OR = 2.56; $\chi^2 = 10.527$ on 1 d.f., $p = 0.001$; Fig. 3). The application of fertilizer affected plant survival negatively (OR = 0.10; $\chi^2 = 37.093$ on 1 d.f., $p < 0.001$; Fig. 3).

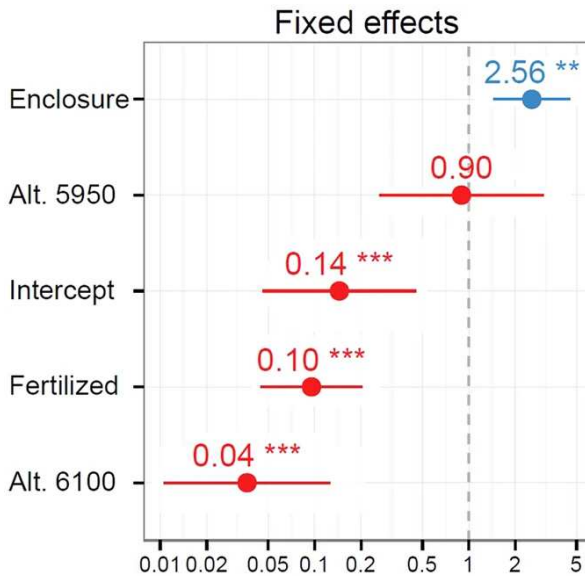


Figure 3. Treatment effects odd-ratios on plant survival. Stone enclosure (Enclosure) shows a significant positive effect, while fertilization had a strong negative effect. Survival at edge site (Alt. 5900) was indistinguishable from source site, effect of transplantation to beyond-range site was solely negative (Alt. 6100).

At 5900 m, the mean annual temperature of the ground inside the stone enclosure was 0.94 K higher compared to control plot (Fig. 4). The increase in soil and near-ground temperatures was less pronounced (0.25 and 0.63 K, respectively).

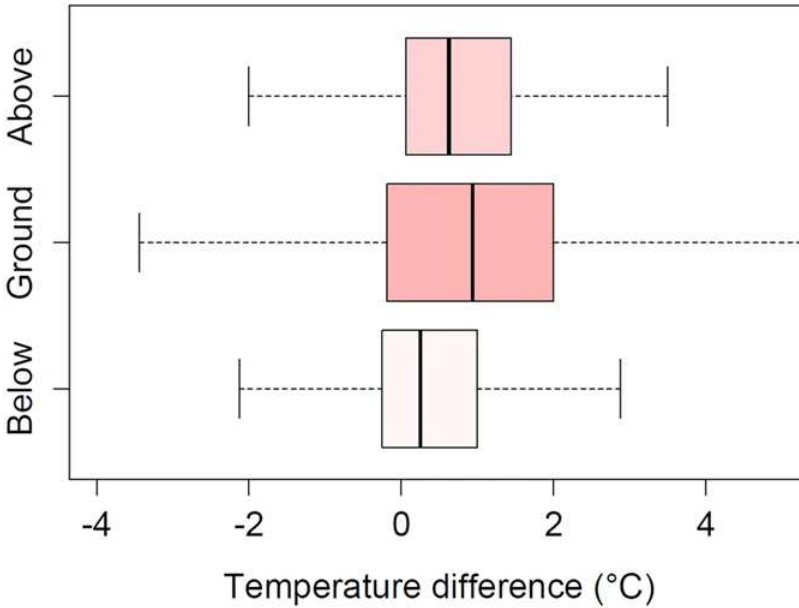


Figure 4. Effect of stone enclosure on microclimate. Differences between sheltered (stone enclosures) and unsheltered plots in below ground (–5 cm), ground, above ground (10 cm) temperatures on the edge-site at 5900 m in 2010. Note that stone enclosures increased the temperature in all three cases.

Discussion

Nine mature individuals of three species survived three years above the regional elevation limit of continuous vegetation. Although far from thriving, those nine plants persisted for three years after their transplantation and went extinct only then. A three-year-long period of persistence is sufficient enough to show that the site was habitable and would imply that some of the studied species have a potential for upward migration. The upward migration, however, will depend on seed production in source populations³⁹, species' dispersal capacities^{2,40} and on the suitability of habitats for seedling establishment^{41,42}. Therefore, the response of species to climate change at the upper limit will be species-specific^{43,44}.

Our finding contrasts with the results of an earlier transplant experiment from the region⁸, performed in 2001, where plants were able to survive only at

the elevation of their natural maximum occurrence. Klimeš and Doležal⁸ concluded that this elevation (~6000 m) was limiting for vegetation and the higher altitudes were uninhabitable for vascular plants due to ecophysiological constraints resulting from the non-existence of suitable habitats. It is likely that the three-year-long survival on our beyond-range site is connected to the warming of the region. Large-scale warming trends and predictions announcing further warming seem to be univocal for this region in a broad sense (NW Himalaya, W Tibet), and the *in-situ* temperature measurements taken were in accordance with these prognoses. The mean temperature of summer months keeps rising and this fact alone would support an upward shift of vegetation, similar to adjacent parts of the Himalayas⁴⁵. The stone enclosures simulated the natural situation where plants in these extreme elevations occur in the shelter of larger stones which provide similar benefits. Nevertheless, the plants in our study were also able to survive in the unsheltered plots which shows their ability to survive on exposed ground without shelter. Interestingly, in 2010, we discovered an isolated outpost site at 6150 m colonized by five species of vascular plants (Table 2). This site was microclimatically exceptional-facing southwest and being sheltered by large stones accumulating heat, the site had microclimate similar to sites in substantially lower elevations, e.g. the edge transplant site at 5900 m (Table 1). This finding nicely illustrates the fact that the elevation per se is not the key driver behind the upper limit of vascular plants⁶.

The fertilizer amendment decreased the probability of survival, even if the nutrient release in this arid climate was exceptionally slow and the tablets were not yet fully dissolved after three years in 2012. This supports the idea of sink-limitation in high-altitude plants which are unable to utilize extra nutrients due to limiting effects of low temperatures on enzymatic systems⁶.

Regardless of the elevation, the best surviving species were *Poa attenuata*, *Saxifraga cernua* and *S. nanella*. These three species have in common their adventive root system and epigeogenous rhizomes⁴⁶. Under the challenging condition of everyday freezing and thawing of the substrate, such a root system may prove advantageous in comparison to a tap root. *Saxifraga cernua* has the potential for fast, vegetative spreading due to its detachable bulbils located in leaf-axils. In one case, it established a new plantlet from the detached bulbil on the edge-site in 2010, moreover two detached bulbils were found on the beyond-range site in 2011. This fact is especially interesting given that *Saxifraga cernua* had the natural upper limit at the lowest elevation (5890 m; Fig. 5) of all of the transplanted species and thus seems more dispersal-limited at this elevation than others. The only species which bore flowers in 2012 at the edge-site were likewise *Poa attenuata*, *Saxifraga cernua* and *S. nanella*, a fact which illustrates their success. However, no plants flowered at the beyond-range site.

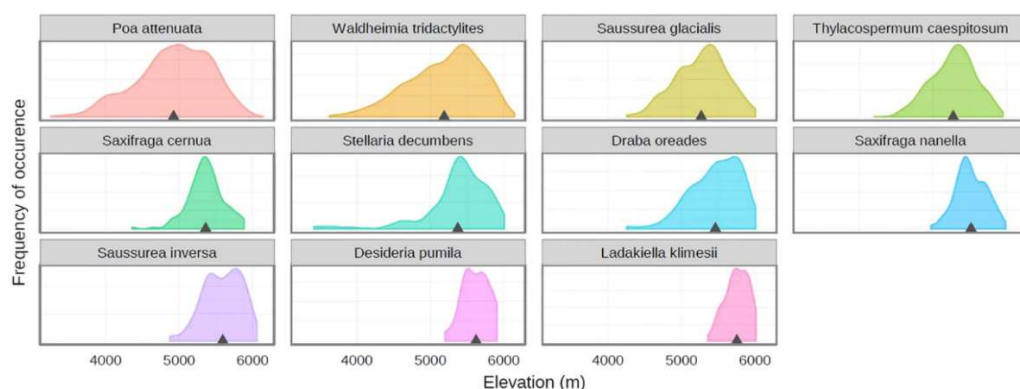


Figure 5. Elevational ranges of 11 species transplanted beyond their local elevational maximum. Species are arranged according to the increasing elevation optimum. Black triangles depict the mean elevation of localities where the species is present.

Our results bring new insight into the minimum heat requirements of vascular plants. So far, the lowest sum of heat recorded at a place with vascular plants was 121 degeedays above 0 °C in the root-zone (Himalaya, 6030 m)⁸, followed by 178 degeedays reported from 4545 m in the Alps (Körner⁷). Klimeš and Doležal⁸ found the upper elevational limit of vascular plants (*Poa attenuata*

and *Waldheimia tridactylites*) at 6030 m on the western slope of Chalung Peak, situated 10 km north of our study site; their measurements in 2002–2003 showed the growing season to be about 50 days long with seasonal mean root-zone temperature as low as 1.7 °C. The presence of vascular plants at this locality, however, was not confirmed in 2014, and the site was completely covered by a thick layer of frozen snow during the whole vegetation season in 2015. The coldest sites in our experiment, where vascular plants naturally occurred, were those at 5900 m and 6150 m; both sites accumulated 163 degreedays above 0 °C. This suggests that the minimum growing season length for persistent vascular plant life is about 60 days, with seasonal mean root-zone temperature 2–3 °C⁷, which results in the sum of heat greater than ca. 160 degreedays above 0 °C. Interestingly, both uppermost plant localities with available microclimatic data (Chalung Peak 6030 m and Shukule Peak 6150 m) have in common a period of at least four weeks without frost in the root-zone, despite their difference in absolute elevations and seasonal mean temperature (1.7 °C vs 2.8 °C). This suggests that the root-zone temperature is more important determinant of upper elevational limit of vascular plants than other commonly used climatic measures, e.g. mean ambient temperature. The freezing of the soil is more critical for vascular plant survival than the above-ground frost because rhizodermis of roots represents a less effective barrier against ice-nucleation when in contact with external soil ice⁴⁷.

The variation in the upper limit among species was found to be fairly low, not broader than ca. 100 m of elevation. A similar finding was reported by Dvorský *et al.*¹⁰ who focused on the upper elevation limits of subnival species in the region; 16 species out of the 24 reaching above 5800 m had their upper limit within 100 m of 6000 m, with a steep decline towards the higher elevations. In our experiment a relatively small increase in elevation from 5900 to 6100 m limited the survival of most transplanted plants. Such a sharp boundary reflects a strong limitation through temperature-related constraints at these range margins, resulting in asymmetric (skewed) distribution curves⁴⁸. Additionally, the steep vegetation decline at ca. 6000 m is further reinforced by a physical

constraint caused by terrain morphology. The elevation of 6000 m is right at the point where the gentle plateau slopes steepen; where the protruding windswept peaks start raising. This determines an abrupt change in microclimate. While on the gentle slopes the temperature is steadily decreasing, there is a sudden jump at 6000 m. Indeed, the adiabatic lapse rate of atmospheric temperature, which in Himalaya ranges 0.5–1 K per 100 m from humid to arid regions⁶, corresponds relatively well to the overall difference in mean temperature of the vegetation season of the transect. However, between 5750 and 5900 m sites the temperature gradient is rather moderate (0.6 K per 100 m in 2009 and only 0.33 K per 100 m in 2010), while the difference between 5900 m and 6100 m was greater than expected (1.0 and 1.5 K per 100 m in 2009 and 2010, respectively) and can be ascribed to the glacier proximity and increased exposure to strong winds at the upper site. Such strong microclimate deterioration was also noticed by Klimeš and Doležal⁸, who inferred from their measurements that the difference in the vegetation season length between their transplant sites at 6030 m and 6160 m was twofold, together with frequent freezing of the soil on the upper site. Another reason for the sharp upper limit is that the proportion of patches with suitable substrate compared to uninhabitable screes considerably drops, so that the distances for dispersal are larger and the overall probability of diaspores to reach a suitable microsite decreases, a factor which can itself result in range limits if microsite colonization rates are lower than extinction rates⁴⁹.

Conclusion

In the first three years of our experiment (2009–2012), there were plants surviving beyond the regional upper limit of vegetation. This supports the hypothesis of dispersal and/or recruitment limitation. Significant warming, recorded *in-situ* during this period, very likely played an important role in the survival. The survival, however, was ecologically non-random process and species better adapted to repeated freezing and thawing of the soil survived

significantly better. No species have survived above the actual vegetation limit since 2013, which we ascribe to the extreme snowfall in 2013. Thus, our experiment sheds light on the complex dynamics of vegetation at its absolute elevational limit where only a small shift in microclimate, e.g. caused by an episodic climatic event, determines whether a population survives or dies off. Our results show that slope exposure and micro-topography are crucial for a successful colonisation and, on the local scale, may be more important than elevation as such.

We conclude that the current elevational limit of vascular plants in the region is maintained by the minimum heat requirements of species, accompanied by a combination of limitations in seed production, dispersal and recruitment. Episodic climatic events also play a great role because of their immediate impact. Considerable warming of the region as well as individualistic responses of the species support the idea that the composition of high-elevation plant communities might change in the future, though the change is not presumed to be fast, given the slow-growing nature of species, their ability to persist even under adverse conditions, and the limitations in propagation.

Methods

Study region

The study was conducted in Changthang region in East Ladakh, Jammu & Kashmir state, NW India. It represents the southwesternmost extension of the Tibetan Plateau with relatively gentle slopes and several peaks exceeding 6500 m. Experiments were performed on the north slope of Chamser Kangri Peak (6622 m a.s.l.), located east of Tsomoriri Lake at the end of the Lupgo valley (N 32° 59.933' E 78° 26.601'). The climate is arid (ca. 100 mm yr⁻¹), most precipitation falls during summer, and above ca. 5000 m practically only snowfall occurs. Winter precipitation is erratic; snow pack is usually thin and discontinuous within the region⁵⁰. The temperature regime is characterized by

a large diurnal range¹⁰. Bedrock is composed of gneiss; soils are characterised by their relatively fine-grained structure, high pH (7.5–9), relatively high concentrations of macronutrients (Supplementary Table S3), and with low organic matter content⁵¹. High pH values of the substrate are caused by alkaline salts which are widespread in the region. Biological soil crusts are a typical feature on the surface of subnival soils⁵². Vegetation is continuous, although sparsely scattered, up to 5950–6000 m, but isolated micropopulations on exceptionally favourable microsites can be found even higher¹⁰.

Experiment

In 2008–2009 we mapped the upper elevation range of subnival species between Chamser Kangri and Shukule Peaks. In addition, we compared our findings with the results of floristic mapping carried out in 2001–2003 within a wider region by our late colleague Leoš Klimeš.

On 12 August 2009, we transplanted 11 common subnival species (Table 2) from the core of the subnival zone (5750 m) to a nearby site at the same elevation (source site, control), to the edge of the vegetation range (edge site, 5900 m) and beyond it (beyond-range site, 6100 m; Fig. 1). The target species represented different growth and functional types of plants⁴⁶ (loose to compact cushions, rhizomatous, rosette or turf plants, clonal and non-clonal). They were randomly selected from the core of the subnival zone, removed as carefully as possible with the soil attached to the roots and transplanted on the same day. We applied two treatments in full factorial design, resulting in four plots at each of the three transplants sites: 1) control plot, 2) stone enclosure, 3) fertilizer addition and 4) stone enclosure and fertilizer addition (Supplementary Fig. S4). The two plots with fertilizers were never located upslope from the non-fertilized plots to avoid contamination. There were 8 rows within each plot, each row contained 11 species randomly mixed, with individuals planted ca. 15 cm apart. In total, we transplanted 1056 individuals (3 altitudes × 4 plots × 8 rows × 11 species). We built enclosures made of stones ca. 40 cm in size (Fig. 6) in an attempt to ameliorate the conditions, specifically to increase the temperature



Figure 6. Slope with experimental sites (left), treatment plots within the edge transplant site at 5900 m (right).

and protect against wind, and buffer temperature oscillations. Silvamix® (Ecolab Znojmo, Czech Republic) fertiliser tablets with slow nutrient release (NPK with trace elements) were used. One tablet was placed ca. 3 cm from each plant, resulting in a total amount of 80–100 kg N ha⁻¹ yr⁻¹.

Microclimatic loggers were placed at each site. Air humidity and soil surface temperature were recorded by R3120 loggers (Comet System, Brno, Czech Republic). Two iButton® loggers DS1921G (Maxim Integrated, San Jose, USA) recorded soil temperature 5 cm below the surface; one logger was inside the stone enclosure, the other one in the control plot. The air temperature at 10 cm above soil surface was recorded by TMS loggers (TOMST®, Prague, Czech Republic). In addition, HOBO® logger (Onset, Bourne, USA) was recording the air temperature and relative air humidity for the duration of the experiment (2009–

2015); it was placed at 5900 m, ca. 1 km off the experimental sites 30 cm above ground, shaded. Due to an unfortunate series of troubles with microclimatic loggers (discontinuous records) which made basic comparisons among sites and treatments impossible, we had to introduce other TMS loggers in 2013 (source site at 5750 m, edge site enclosure at 5900 m, beyond-range site enclosure at 6100 m). Growing season length was calculated as number of days with mean soil temperature above zero^{7,8}.

At each transplanted plant, we were measuring annual growth (size of leaf-rosette, height, number of shoots, number of flowers) for three years at the end of vegetation season until the final assessment in September 2012. From 2013 to 2015, we made only cursory examinations of the transplant sites, particularly to check the beyond-range site for surviving individuals.

Functional differences between species

We used a database of functional traits of high-altitude plants in Eastern Ladakh by Chlumská⁵³ (with the methods of data collection therein; see also Cornelissen *et al.*⁵⁴) in order to reveal if any of the traits can explain differences in survival of the transplanted species. We employed plant height, growth form⁴⁶, leaf and stem dry matter content (LDMC and StDMC), C:N ratio, seed mass, leaf nitrogen and phosphorus concentrations (LNC, LPC), leaf $\delta^{13}\text{C}$, root nitrogen and phosphorus concentrations (RNC, RPC), non-structural soluble carbohydrates (NSC; starch, fructans, raffinose family oligosaccharides, simple sugars and sugar alcohols). The traits were measured in a minimum of 10 individuals (up to 50 for some species) from various elevations and the average values were calculated per species.

Data analysis

To explore the general effects of experimental treatments, we analysed the data on transplanted plants by generalized mixed effect models. Mortality in the first year of experiment could be induced by direct plant damage due to transplantation, therefore we ran analyses only on second-year survival. We

used the *glmer* function with logit link function from R package *lme4*⁵⁵. We tested the significance of model terms by type II Wald χ^2 test. Wald z statistics were used to test the significance of treatment levels. We obtained effect odds ratios (OR) and visualized models by *sjp.glmer* function from R package *sjPlot* (Lüdecke 2015). We tested the effects of experimental treatments (site elevation, enclosure, fertilization) on survival as fixed effects, while species identity was considered as a random effect. We expected a species-specific response to experimental treatments beyond their elevational range, therefore we used the Akaike information criterion (AIC) to compare the performance of the model with only random intercepts for each species and the model with random effects on each elevation level for each species. The model with lower AIC was selected. To interpret these random effects, we searched for correlation between species functional traits and corresponding random effects. We applied Bonferroni correction to model ANOVA p-values to correct for multiple testing (p_{corr}). The analyses were performed in R (R Development Core Team 2015).

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Author information

Contributions

J.D., M.D. and Z.C. designed the study. M.D., Z.C., J.A., K.Č., K.Ř., M.M., M.K. and J.D. collected field data. M.M., Z.C., J.D., M.D. and M.K. performed statistical analyses. M.D., Z.C., J.A., J.D. and P.L. wrote the first draft of the manuscript, and all authors contributed substantially to revisions.

Ethics declarations

Competing interests

The authors declare no competing financial interests.

Supplementary information

Supplementary Table S1. Trends in mean daily temperature of respective months. Linear regression equation, regression coefficient (R^2) and level of significance (P; *** $P < 0.001$; ** $P < 0.01$; * $P < 0.05$) are shown.

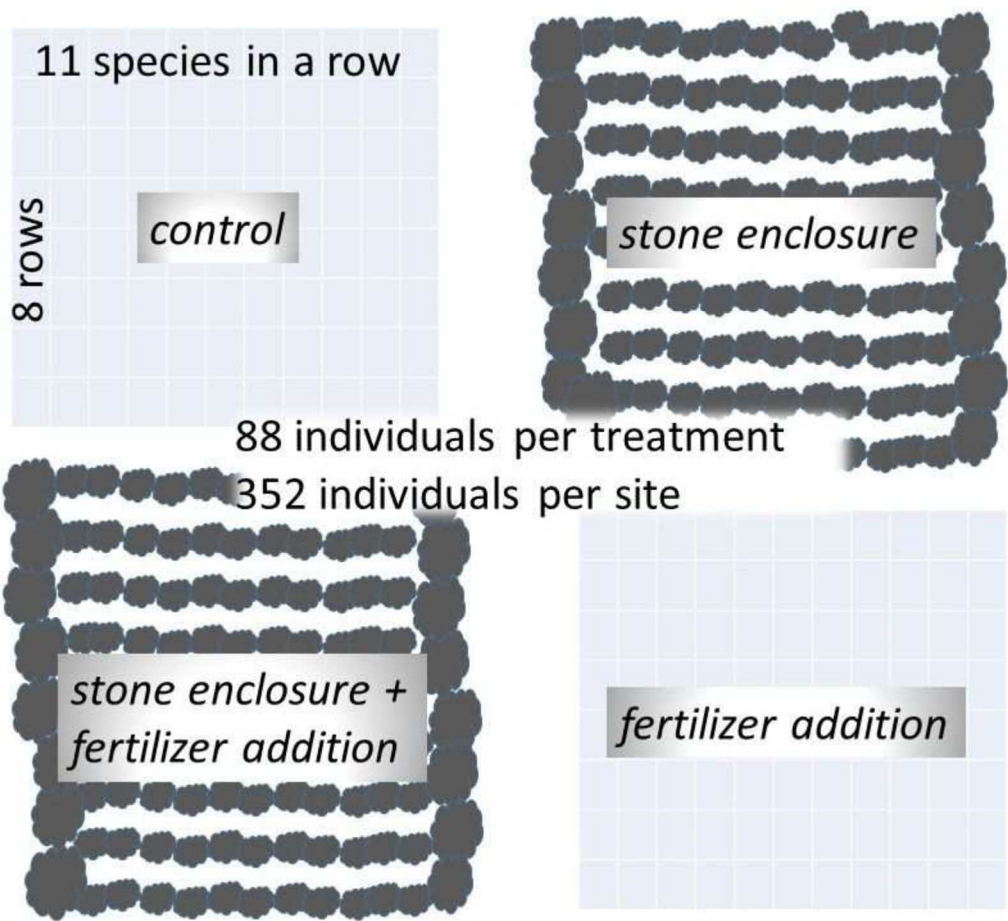
Month	Linear Regression	R^2	P
January	$y = -0.4803x + 943.73$	0.307	0.254
February	$y = -0.3852x + 753.92$	0.67	0.046*
March	$y = -0.1389x + 261.3$	0.308	0.253
April	$y = -0.1452x + 278.15$	0.041	0.699
May	$y = 0.2697x - 551.29$	0.068	0.618
June	$y = 1.2272x - 2471.8$	0.709	0.035*
July	$y = 0.5909x - 1186$	0.821	0.013*
August	$y = 0.1172x - 233.65$	0.184	0.337
September	$y = 0.69x - 1390$	0.795	0.007**
October	$y = 1.0713x - 2165$	0.562	0.085
November	$y = 0.2028x - 423.9$	0.052	0.665
December	$y = 0.0861x - 192.97$	0.047	0.68

Supplementary Table S2. Growth and flowering of plants on the transplant sites at 5950 and 6100 m a. s. l. Values represent per cent change in size, number of shoots, height, and change in the number of flowering shoots from 2010 to 2012. Size (%) – per cent change in the size (length*width of the basal part of an individual) of plants; Shoot (%) – per cent change in the total number of shoots; Height (%) – per cent change in the height of the highest sterile shoot; Flowering shoots – change in the number of flowering shoots (e.g. 0 = the plant never flowered or the number of flowers remained the same; 1 = the plant had one more flowering shoot in 2012 compared to 2010; -2 = the plant had two flowering shoots fewer in 2012 compared to 2010). p – significance from one sample t-test (H0 = 100 %, i.e. plant didn't grow), * p< 0.05 , **p<0.01, *** p<0.001, ns – non-significant. No – number of individuals.

treatment	Change from 2010 to 2012											
	Size (%)	p	No	Shoots (%)	p	No	Height (%)	p	No	Flowering shoots	p	No
5900 m a. s. l.	132.6	ns	37	74.2	.	32	122.6	ns	48	1.3	.	59
fertilized	148.1		1	40.0		1	285.7		1	6.0		1
<i>Poa attenuata</i>	148.1		1	40.0		1	285.7		1	6.0		1
control	51.7		12	39.1	**	10	106.3	ns	15	0.1	ns	24
<i>Poa attenuata</i>	33.8		4	44.0	ns	2	75.0	ns	5	0.5	ns	8
<i>Draba oreades</i>	11.9		1	16.0	.	2	125.0	ns	2	0.0		2
<i>Saxifraga cernua</i>	130.3	ns	2	95.8	ns	2	145.0	ns	2	-0.7	ns	3
<i>Stellaria decumbens</i>	55.7		1				250.0		1	0.0		2
<i>Saxifraga nanella</i>	42.5	ns	2	16.6	ns	2	100.0		2	0.0		4
<i>Thylacospermum caespitosum</i>	35.7	ns	2				50.0		1	0.0		2
<i>Waldheimia tridactylites</i>				23.2	ns	2	90.0	ns	2	0.0		2
enclosure	99.9	ns	18	92.6	ns	18	123.7	ns	26	0.6	ns	28
<i>Poa attenuata</i>	108.4	ns	8	300.0		1	116.4	ns	8	3.0		8
<i>Ladakiella klimesii</i>										0.0		1
<i>Desideria pumila</i>				33.0	ns	3	150.0	ns	3	-2.0	ns	3
<i>Saxifraga cernua</i>	174.9	ns	4	135.7	ns	7	152.8	ns	7	-0.1	ns	7
<i>Stellaria decumbens</i>	30.8	ns	2				150.0		1	0.0		2
<i>Saussurea glacialis</i>	48.0		1	100.0		1	66.7		1	0.0		1
<i>Saxifraga nanella</i>	40.9	ns	3	36.4	*	6	91.7	ns	6	0.2	ns	6
fertilized and enclosure	389.6	ns	6	91.4	ns	3	131.2	ns	6	8.5	ns	6
<i>Poa attenuata</i>	171.5	ns	3				69.7	ns	3	13.3	ns	3
<i>Saxifraga cernua</i>	607.7	ns	3	91.4	ns	3	192.7	ns	3	3.7	*	3
6100 m a. s. l.	16.0	*	4	48.1	*	9	34.5	**	6	-0.1	ns	9
fertilized	6.7		1	28.6		1	20.0		1	-1.0		1
<i>Saxifraga cernua</i>	6.7		1	28.6		1	20.0		1	-1.0		1
control				50.0		1				0.0		1
<i>Saxifraga cernua</i>				50.0		1				0.0		1
enclosure	19.2	.	3	50.6	.	7	37.4	*	5	0.0		7
<i>Poa attenuata</i>	0.7		1	15.0		1	46.7		1	0.0		1
<i>Saxifraga cernua</i>	53.3		1	75.6	ns	4	24.5	*	3	0.0		4
<i>Saxifraga nanella</i>	3.5		1	18.3	.	2	66.7		1	0.0		2

Supplementary Table S3. Soil qualities at transplant sites. Numbers represent averages from 12 soil samples per site. Soil texture means the proportion (%) of soil particles bigger than 0.5 mm.

	5800 m	5950 m	6100 m
Soil texture	2.7	6.6	4.4
pH	7.8	8.7	9.1
TN [mg/kg]	1906	1072	941
P-PO ₄ ³⁻ [mg/kg]	44	227	172



Supplementary Figure S4. A top view sketch of the experimental arrangement.

CHAPTER III

How to preserve plant samples for carbohydrate analysis? Test of suitable methods applicable in remote areas

Zuzana Chlumská, Štěpán Janeček and Jiří Doležal



Chlumská, Z., Janeček, Š., Doležal, J., 2014. How to preserve plant samples for carbohydrate analysis? Test of suitable methods applicable in remote areas. *Folia Geobotanica* 49, 1-15.

Photo on previous page: NSC sample preservation method and necessary equipment in practice in Ladakh.

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(Published)

Abstract

Nonstructural carbohydrates (NSC) are frequently studied both by ecologists and plant physiologists because they serve as fundamental carbon and energy sources in plant metabolism. Because of the rapid enzymatic hydrolysis of NSC after harvest, plants are usually placed into liquid nitrogen until subsequent analyses in the laboratory. Nevertheless, when the research is performed in poorly accessible regions such as high mountains, tropical forests or deserts, the use of heavy containers containing vaporizing liquid nitrogen is problematic for logistical reasons. These places are particularly interesting as they harbor plant species with interesting physiological adaptations. In our study we aimed to develop a suitable storage method for plants intended for NSC analyses, which would require minimal equipment. These demands resulted in the idea of using the first step in NSC analyses – extraction in ethanol. Samples were extracted in 50 % and 96 % boiling or non-boiling ethanol and then stored for one month; they were compared with samples immediately frozen in liquid nitrogen. We discovered that for samples containing starch, fructans, soluble sugars and sugar alcohols, the best pretreatment for subsequent storage is extraction in 50 % or 96 % boiling ethanol. For total nonstructural carbohydrates (TNC) assessment, only extracting with 50 % ethanol without boiling gave very good results. Finally, we developed a method that could be used in any remote place without bulky laboratory equipment.

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CHAPTER IV

Species- and compound-specific dynamics of nonstructural carbohydrates toward the world's upper distribution of vascular plants

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Photo on previous page: some of the species examined in the article, from top left to right – *Oxytropis microphylla*, *Elymus schrenkianus*, *Delphinium brunonianum*, *Arenaria bryophylla*, *Ladakiella klimesii*, *Waldheimia tridactylites*, *Poa attenuata* and the extracts used for the NSC analyses

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Abstract

Nonstructural carbohydrates (NSC) are the crucial components of plant metabolism, and NSC dynamics provide essential information on plant carbon balance, which usually changes toward the distribution limits of plants. NSC have been extensively used to study the elevational limit in trees all around the world. For alpine herbs, which occupy vast areas beyond treelines, such investigations are still lacking.

To assess whether carbon supply is a limiting factor for the upper distribution limits of alpine herbs, we studied NSC in belowground organs of 11 perennial species across their 4500–6000 m elevation ranges in the Western Himalayas at the peak of the growing season.

We found a general elevational increase in total NSC within and across species. Studied alpine plants contain simple sugars and fructans as the main NSC and less so starch. Species- and compound-specific elevation patterns were found for different NSC compounds, suggesting a regulatory role of NSC in alpine herbs acclimation rather than a general accumulation of all NSC resulting from growth limitation.

Similar to studies on treeline NSC dynamics, our study shows that reduced carbon supply is an unlikely cause of high-elevation range limits in herbs. Yet, the elevational NSC dynamics in alpine herbs still needs further exploration,

CHAPTER IV

with an emphasis on different compounds and their function in acclimatory metabolic processes, to clearly identify the key mechanisms determining plant range limits.

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SUMMARY OF RESULTS

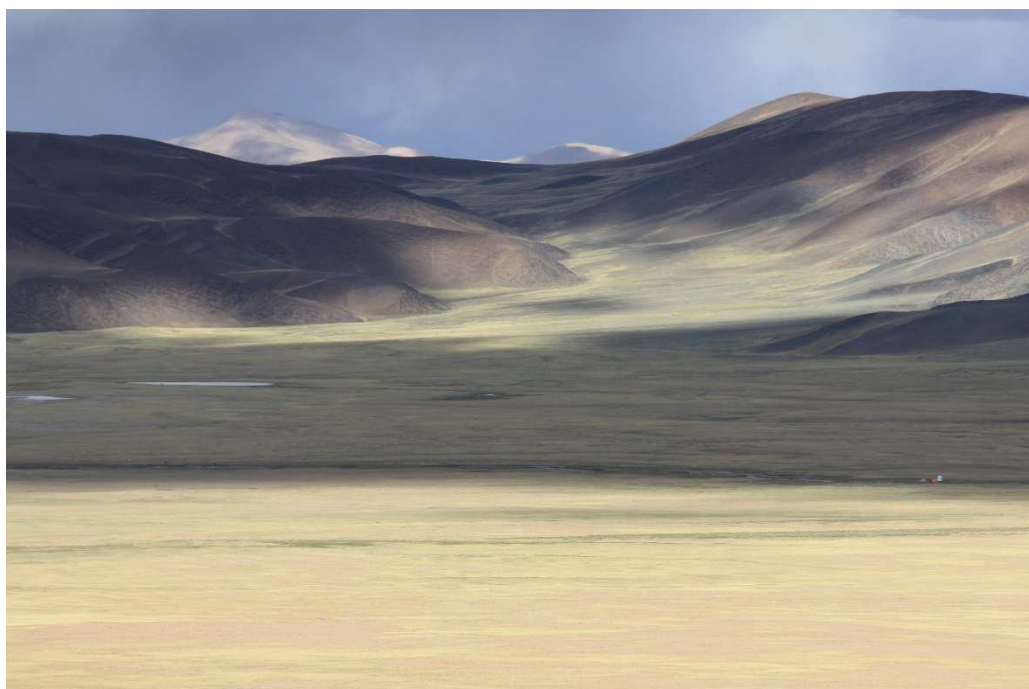


Photo on previous page: high elevation steppes of Rupshu region, Ladakh.

Summary of results

The main goal of my dissertation was to investigate the vegetation dynamics, species characteristics, and the main limiting factors affecting plant performance at the world's elevational frontier of vascular plant distribution (**Chapter I, II, IV**). This work may help to more accurately predict the future direction of changes in the alpine environment linked to global climate change, at least in the study region, if not in general.

We observed an upward shift of subnival species in relation to higher summer temperatures, which was also confirmed by plant age analysis. However, we also found that higher precipitation in the study region increased the incidence of deleterious soil freeze-thaw events. This is especially dangerous for seedlings, but can probably damage or even uproot adult plants. This may be the cause of the decline in the abundance of subnival species in the permanent plots monitored, as well as a shift in species composition towards plants preferring wetter habitats, which are probably better adapted to this process. These species are often clonal and have adventitious roots. Our results suggest “*hygrophilization*” of the highest growing plant communities in the region rather than *thermophilization*. These important findings, presented in **Chapter I**, suggest that we can expect multidirectional species- and site-specific changes in the future, in addition to the temperature-driven upward shift of plants in the alpine environment.

The transplant experiment we conducted at and beyond the regional elevational frontier of continuous vegetation cover (**Chapter II**) provided even more insight into the factors affecting the performance of the highest-occurring plants. Our experiment showed that some species are able to grow at higher elevations, supporting the hypothesis that upward movement is probably prevented by dispersal and/or recruitment limitations. The key factors determining survival turned out to be the temperature of the root zone above 0 °C and the sums of heat represented by degree days above 0 °C. The warmer environment provided by stone enclosures led to higher survival rates,

confirming that microtopography, and thus microclimate, plays a crucial role in high-elevation habitats. This also suggests that the presence of suitable habitats at higher elevations, such as large heat-accumulating boulders, may be important for the colonization of unglaciated areas. However, plants were also able to survive in unprotected plots, even in habitats beyond the limit of regional vegetation, but with a lower success rate. Nutrient limitation does not appear to be the cause of elevational distribution limits as nutrient supplementation did not increase survival rates. Interestingly, similar to the study in **Chapter I**, we found that plants with adventitious roots, which are probably better adapted to the soil freeze-thaw cycle, had a greater chance of establishing. Importantly, we observed that unpredictable episodic extreme events, such as the massive snowfall in 2013, can overcome these advantageous species characteristics and kill all plants. However, the lack of detailed long-term climate data, especially on extreme precipitation in the study area, does not allow us to make any predictions.

Carbon limitation is unlikely to be the cause of the upper limits of plant distribution at high elevations, as we found no depletion of NSC towards higher locations. These results are presented in **Chapter IV**. We also found that these plants are relatively high in sugars and fructans compared to starch. However, the reason for such composition, as well as the generally higher NSC levels with elevation and the diverse elevational patterns of different NSC compounds, still remain to be revealed. We believe that it is not the lack of carbon compounds, but rather the function of NSC in acclimation to the harsh environment that may be important for distributional limits (the results of **Chapter III** were necessary to carry out this study).

Overall, we conclude that future climate change-related vegetation changes in our study region of Ladakh are likely to be influenced by a combination of changes in temperature and soil moisture, and will be species-specific and more complex than a simple upward shift of plants towards deglaciated areas, as is often assumed.

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EDUCATION

2005 – up to now University of South Bohemia in České Budějovice, Faculty of Science, Czech Republic

2005-2008: Bc. in Biology, thesis: *Common ash (Fraxinus excelsior) expansion in the Vyšenské kopce National Nature Reserve.*

2008-2011: Mgr. in Vegetation Ecology, thesis: *Functional traits of high-altitude plants in Eastern Ladakh (NW Himalayas).*

2011-present: Ph.D. study programme Botany

EMPLOYMENT

2010-2013: Institute of Botany, Třeboň, Functional Ecology department, Ph.D. student

2013-2019: Parental leave

2020-present: Institute of Botany, Třeboň, Functional Ecology department, Technical Assistant

RESEARCH EXPERIENCE AND INTERSHIPS

2008-2012, 2022 – six sampling expeditions in Ladakh

2013 – WSL Institute, Birmensdorf, Switzerland; laboratory of Fritz Schweingruber. Sectioning and staining of belowground plant organs brought from Ladakh for growth and age analyses.

2020 – Department of Biogeochemical Processes, Max Planck Institute for Biogeochemistry, Jena, Germany; a group of Henrik Hartmann. Sample preparation for the HPAEC-PAD and collaboration on the Chapter IV article.

CONFERENCE PRESENTATIONS

- 2009 – Poster at PopBio conference (Plant Population Biology in a changing world) in Bern: “*Nutritional status of high-altitude plants in Ladakh, NW Himalayas.*”
- 2013 – Oral presentation at 16th IALS (International Association for Ladakh Studies) conference in Heidelberg: „*Can plants grow higher?*”

PUBLICATIONS (NOT PART OF THE PH.D. THESIS)

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SCHOLARSHIPS

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