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Ecology of alpine plants in NW Himalaya

Ph.D. Thesis

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ANNOTATION

The westernmost spur of the Tibetan Plateau stretches to Eastern Ladakh in India. It is a region which remains poorly explored because of challenging conditions and long periods of political instability. At the same time, it is one of the highest places on earth supporting angiosperm life, which goes beyond 6000 m a.s.l. here. The whole region, due its remoteness, is practically unaffected by plant invasions and direct human activities. Thus, Ladakh represents a kind of “natural experiment”, providing very long gradient of elevation suitable for comparative functional ecology as well as for testing various hypotheses concerning limitations of vascular plants. Arid climate and extreme elevations are the common factors.

Our team pursued the goal of systematic botanical and ecological exploration of Ladakh, started by late Leoš Klimeš. This thesis provides insight into the main vegetation types, clonality in plants, plant-plant interactions and soil phototroph communities.

DECLARATION [IN CZECH]

Prohlašuji, že svoji disertační práci jsem vypracoval samostatně pouze s použitím pramenů a literatury uvedených v seznamu citované literatury. Prohlašuji, že v souladu s § 47b zákona č. 111/1998 Sb. v platném znění souhlasím se zveřejněním své disertační práce, a to v úpravě vzniklé vypuštěním vyznačených částí archivovaných Přírodovědeckou fakultou elektronickou cestou ve veřejněpřístupné části databáze STAG provozované Jihočeskou univerzitou v Českých Budějovicích na jejích internetových stránkách, a to se zachováním mého autorského práva k odevzdanému textu této kvalifikační práce. Souhlasím dále s tím, aby toutéž elektronickou cestou byly v souladu s uvedeným ustanovením zákona č. 111/1998 Sb. zveřejněny posudky školitele a oponentů práce i záznam o průběhu a výsledku obhajoby kvalifikační práce. Rovněž souhlasím s porovnáním textu mé kvalifikační práce s databází kvalifikačních prací Theses.cz provozovanou Národním registrem vysokoškolských kvalifikačních prací a systémem na odhalování plagiátů.

Třeboň 22.1.2014

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Miroslav Dvorský

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LIST OF PAPERS AND AUTHOR'S CONTRIBUTION

The thesis is based on the following papers:

Dvorský M, Doležal J, de Bello F, Klimešová J, Klimeš L (2011): Vegetation types of East Ladakh: species and growth form composition along main environmental gradients. *Applied Vegetation Science* 14: 132-147 (IF=2.263).
Miroslav Dvorský was responsible for data assembly, writing the manuscript and partially for statistical analysis.

Klimešová J, Doležal J, **Dvorský** M, de Bello F, Klimeš L (2011): Clonal growth forms in eastern Ladakh, Western Himalayas: classification and habitat preferences. *Folia Geobotanica* 46: 191-217 (IF=1.565).
Miroslav Dvorský participated in data assembly, writing and revision of the manuscript.

de Bello F, Doležal J, **Dvorský M**, Chlumská Z, Řeháková K, Klimešová J, Klimeš L (2011): Cushions of *Thylacospermum caespitosum* (Caryophyllaceae) do not facilitate other plants under extreme altitude and dry conditions in the north-west Himalayas. *Annals of Botany* 108: 567-573 (IF=3.449).
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Dvorský M, Doležal J, Kopecký M, Chlumská Z, Janatková K, Altman J, de Bello F, Řeháková K (2013): Testing the stress-gradient hypothesis at the roof of the world: effects of the cushion plant *Thylacospermum caespitosum* on species assemblages. *PLoS ONE* 8: e53514 (IF=3.730).
Miroslav Dvorský participated in experiment preparation and data collection in the field and was responsible for data assembly, writing the manuscript and partially for statistical analysis.

Janatková K, Řeháková K, Doležal J, Šimek M, Chlumská Z, **Dvorský M,** Kopecký M (2013): Community structure of soil phototrophs along environmental gradients in arid Himalaya. *Environmental Microbiology* 15: 2505-2516 (IF=5.756).
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Dvorský M, Doležal J, Kopecký M, Chlumská Z, Altman J, Řeháková K, Janatková K: Vegetation of extreme elevations: a case study from subnival zone of E Ladakh, Transhimalaya. (*submitted manuscript*).
Miroslav Dvorský participated in experiment preparation and data collection in the field and was responsible for data assembly, writing the manuscript and partially for statistical analysis.

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CHAPTER I

General introduction

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

General introduction

The focus of my thesis is fairly wide as it covers most results of our team work in Ladakh, western Himalaya, northwestern India. Our activities in Ladakh began in 2008 and followed up with work started by our late colleague Leoš Klimeš. He pursued a goal of a complete flora of the region so that systematic floristic mapping was his ultimate activity. Besides, Klimeš was interested in clonality of plants and carried out experiments concerning limitations of plants at their upper distributional limit. His results and knowledge enabled us to begin with research in a region we all were strangers to. We could learn plant species from his herbary collections, we could use his great database to gain idea on species distribution. If it were not him, we could hardly start. Since the thesis engages in many topics which have in common the environmental background only, it is hard to generalize the results as such. That is why I comment on the chapters separately and explain their merit in the respective fields. Several studies, however, are simply descriptive and need not a comment, except the fact that they literally filled a symbolic white place on a map. Nevertheless, topics concerning ecological theories and hypotheses having experimental design are presented in a wider perspective.

Study region

From both geomorphological and phytogeographical points of view, Ladakh can be subdivided in three major regions (Dickoré, 1995): Zanskar in the west and south, Ladakh in the north and and Rupshu in the east. The former two parts are dominated by deep and narrow valleys originating as low as 2600 m. Local flora, like that of much of the Karakorum mountains, is distinct and true Himalayan species are more prominent there (Hartmann, 1983, 1990, 1995). It is composed of probably almost balanced proportions of floristic elements from adjacent regions (Central Asiatic, Irano-Turanian including Pamiran in a wider sense and West (Inner) Himalayan. Despite the varied relief and elevation features of that area, the flora seems to be depauperate and composed of accidental elements originating from adjacent regions (Klimeš, unpublished). This is probably due to the comparatively modern and dramatic geological history (Searle et al., 1998), and present-day inhospitable arid conditions (Hartmann, 1983). Since the humidity is continually increasing towards the west and similarly also towards the south, the snow line in the western parts is situated by about 1000 m lower than in the east.

Our team conducted most studies in the eastern part of Ladakh, in Rupshu region. This region is well delimited climatically, biogeographically and also culturally. It covers the south-westernmost extension of the Tibetan Plateau and its rim towards the high-mountain ranges of the Inner Himalaya, including three basins without external drainage. Rupshu represents biogeographically a unique area, located on the interface between the humid outer Himalayan Range and the cold deserts of inner Tibet (Hartmann, 2009). The transitional zone is broad enough here to host a specific flora with dominating Tibetan elements (Dickoré, 1995). Its nearly 20,000 km^2 include a great part of this transitional zone, forming a naturally delimited area of Transhimalaya. Most of the area lies above 4000 m, which is the elevation of the bottom of Indus river Valley, and many peaks considerably exceed the heights of 6000 m (Kapadia, 1999). The relatively flat terrain and vast high-altitude plateau is the most characteristic feature with the snow line at 5800-6000 m. Although precipitation is extremely low (<100 mm), frigid temperatures compensate to a certain degree the effects of continental aridity. The dominating vegetation type is cold alpine steppe. Due to permafrost conditions, and prevailing level glacial and fluvioglacial sediments, water-supply is in places quite sufficient for marshland and turf vegetation. Furthermore there is a variety of substrates ranging from siliceous rocks to calcareous or saline sediments. Hot springs (Puga) occasionally occur on the margin of the area towards the Indus Valley, and contribute to the relatively varied flora.

Flora of the whole Ladakh is undoubtedly Holarctic, with a negligible proportion of Paleotropical elements so that almost all important families and higher taxonomical units of the Holarctic flora are well represented here, except for exclusively woody taxa (Klimeš and Dickoré, 2006). As for the species richness of the region, Klimeš (2003) found about 450 species in his study region in E Ladakh (10,227 km^2). This number is markedly lower than 1000–1500 species per 10,000 km^2 given by Barthlott et al. (1996) for this section of NW India but falls within a range 200-500 species estimated by Dickoré and Miehe (2002) for most of the area. Accordingly, Ladakh has been referred to as a cold spot of diversity, at least in terms of vascular plants (Dickoré and Miehe, 2002).

History of botanical exploration

Foreign explorers reached the area of Ladakh at the beginning of 18th century already, however, first botanical results were obtained by expeditions organised a century later. The most famous names should be mentioned at least, these include W. Moorcroft, H. Strachey, T. Thomson, J. L. Stewart, J. F. Duthie, or the Czech palaeontologist and naturalist F. Stolička. Difficulties with logistics, war conflicts and common problems with obtaining permits from local authorities, however, strongly restricted the explorers and limited the number of plant specimens collected in these remote and wild areas. The results of these early periods were summarised in Flora of India (Hooker, 1875–1897), up to now the only complete standard flora covering the whole Ladakh. The species list of Ladakh flora was published by R. R. Stewart (1916–1917) who compiled all earlier records and added his own data.

Relatively few botanists and collectors visited Ladakh in the course of the first half of 20th century. From the 1940s, Ladakh was closed both for foreigners and Indian botanists for three decades. Botanical research was re-established after the area became partly accessible in the middle of the 1970s. E Ladakh, the area on the focus of this thesis, was opened to foreigners only in 1995. The pioneer of modern vegetation studies in this region was Swiss geobotanist Hans Hartmann. He visited various parts of Ladakh from 1974 to 1997 and contributed substantially to the knowledge of the vegetation composition of the area (see below for details). At the same time Indian botanists have started to explore the areas along the main routes, which resulted in the second flora of Ladakh by Kachroo et al. (1977), Murti's treatment of Monocots (2001) and studies focused on vegetation of the area (Kala and Mathur, 2002; Rawat and Adhikari, 2005). A recent attempt to publish a modern account of flora of NW India is that by Singh et al. (2002) who compiled the first volume of the Flora of Jammu and Kashmir. A part of Ladakh is also covered by the Flora Karakorumensis by Dickoré (1995), which is largely based on specimens deposited in major European herbaria. Some volumes of the comprehensive and still incomplete Flora of Pakistan (Nasir and Ali, 1970–) cover the border areas of Ladakh.

Despite the efforts of many naturalists, starting with many great expeditions in 19th century, there is no precise checklist of Ladakh flora yet, not speaking about a standard exhaustive flora. As a consequence, even basic information such as the number of species in Ladakh remains unknown. This fact can be demonstrated by the discrepancy between estimates of numbers of vascular species in Ladakh, ranging from 611 (Kachroo et al., 1977) and 880 (Kachroo, 1993) to 1250–1500 species (Klimeš, unpublished). This causes serious difficulties not only to scientists, but also to natural protection activities and research focused on medicinal plants, which is a traditional topic of Indian botanists (Kaul, 1997; Chaurasia and Gurmet, 2003).

Klimeš realised the importance of a complete flora and began a proper and systematic floristic exploration of Ladakh in 1997. Even if his task remained unfinished, thanks to his thorough and rigorous attitude the knowledge of Ladakh flora increased significantly. Klimeš discovered many species that were not mentioned from Ladakh before and greatly extended the checklist of species known from this region; the preliminary version of the checklist can be found on internet and includes 1287 taxa (Klimeš and Dickoré, 2006). He also collected several specimens which were recognized as unknown for science and later described (Al-Shehbaz, 2002; Klimeš and German, 2007). Moreover, Klimeš established a herbarium of Ladakh flora which includes more than 7600 specimens and is the largest collection from this region in the world.

Vegetation

Modern phytosociological and floristical explorations were carried out by Hartmann who has done the most detailed work on the vegetation of Ladakh (Hartmann, 1983, 1984, 1987, 1990, 1995, 1997, 1999). His geobotanical description of the region was based on vegetation records and included valuable data on life-form composition as well as soil characteristics. With his 343 vegetation records Hartmann covered the whole Ladakh in terms of the most important ecoregions, including Rupshu. He was, however, focused on zonal vegetation of lower elevations and never worked above ca. 5300 m, omitting the truly unique vegetation of high-alpine and subnival zones. Given the large proportion of vegetated land within these zones, particularly in Rupshu, this meant a vast gap in the knowledge. While vegetation of lower elevations has close affinities to adjacent arid regions where it has already been studied more properly (Dickoré and Nüsser, 2000; Dickoré and Miehe, 2002), the high elevation communities of Rupshu region are more closely related to those of Tibetan Plateau, studied unsufficiently so far (Miehe et al., 2011). Since ecosystems of high-mountain regions in arid Himalaya are expected to be particularly vulnerable to global change drivers their assessment and description is encouraged (Chapters II and VII).

The prevailing arid climate of Rupshu region with annual precipitation below 100 mm is the key factor determining the general feature of the vegetation, while the edaphic qualities and the microclimate govern its local patterns. In general, the vegetation is very scarce and is formed mostly by herb species or low growing shrubs, which makes the characteristic semidesert impression (Hartmann, 2009). Forests are typically absent in the area, except for willow and poplar stands around rivers and irrigation canals. The lowest elevations of E Ladakh are situated in the Valley of Indus River, starting from about 4100 m. The highest peak, Lungser Kangri (6666 m) is devoid of vascular plants, but some species were found as high as 6150 m (Chapter VII). The species density culminates between 4500 and 5000 m and slowly declines towards both ends of the elevation gradient. Eight main vegetation types can be distinguished within the area (Chapter II).

Life forms and clonality

Classification of plant species into easily distinguishable categories with respect to gross morphology has a long tradition, dating back to the attemtps of describing taxonomically unknown vegetation of South America (von Humboldt, 1806). Later, other classification systems were developed or the existing further improved (Grisebach, 1872; Drude, 1886; Warming, 1909; Du Rietz, 1931; Barkman, 1988; Halloy, 1990; von Lampe, 1999). Most of them were based on idea that plants with similar architecture have a similar ecological response to main climatic and edaphic conditions, thus having a predictive value. The Raunkiaer´s concept of life-forms (1910) is the only one to receive wide attention and until today represents a useful tool for description and comparison of vegetation types in different regions with no need of species determination. This quite simple concept of life-forms is strictly based on the location of hibernating or estivating organs in relation to the soil surface. The six basic categories are phanerophytes (trees), chamaephytes (shrubs), hemicryptophytes (perennial herbs with buds near the surface), geophytes (herbaceous plants with an underground storage organ, e.g. bulbs), therophytes (annual herbs which survive unfavourable season in the form of seeds) and hydrophytes (water plants with buds protected in water). Given the specific ecological needs of the respective categories, we can predict the proportion of species belonging to individual life-forms for any type of climate and, conversely, from the information on the life-forms representation we can deduce the prevailing climate. Yet, Raunkiaer´s approach is most usefull in regions with pronounced seasonality where its application brings the most relevant information (Raunkiaer, 1910; Meher-Homji, 1981; Sarmiento and Monasterio, 1983; Campbell and Werger, 1988; Komárková and McKendrick, 1988; Cody, 1989; Danin and Orshan, 1990).

Distribution of life-forms changes with elevation (Berg, 1998; Mark et al. 2000; Pavón et al. 2000; Klimeš 2003). Since elevation as such represents a composed gradient of tightly correlated climatic and edaphic factors (temperature, precipitation, substrate stability, texture, fertility, etc.; Körner, 2003) it is difficult to make any conclusions concerning its effect on life-form spectrum in terms of identifying the concrete responsible variable. Nevertheless, diversity of life-forms usually decreases with elevation and the upper limits are reached by one or two of them, hemicryptophytes in most cases (Dickoré and Nüsser, 2000; Klimeš 2003). The life-form spectrum assessment along the whole elevational gradient in Rupshu region indicates prevailing desert-steppe and steppe vegetation with dominating hemicryptophytes (62.1 %), followed by therophytes (22.3 %), chamaephytes (5.4 %) and geophytes (4.2 %) (Klimeš, 2003). Meanwhile, the diversity of life-forms declines with elevation, phanerophytes and hydrophytes occur up to 5150 m, therophytes decline gradually, geophytes and chamaephytes are constant up to 5800 m, although in low species numbers. Hemicryptophytes are the only life-form at extreme elevations. Similar pattern has been observed in other mountain regions of dry Central Asia, such as Hindukush, Nanga Parbat, Karakorum or Tibetan Plateau (Dickoré and Nüsser, 2000; Dickoré and Miehe, 2002; Baniya, 2010; Baniya et al., 2012). Interestingly, the category of therophytes is highly represented and reaches very high elevation in Ladakh and in adjacent arid regions of Tibet (Banyia, 2010; Banyia et al., 2012). For comparison, therophytes grow up to 5000-5200 m in Hindukush while in Ladakh and Tibet they can be found even above 5600 m.

Clonality in plants is a strategy of multiplying renewing buds and clonal plants are generally abundant in wet, cold and nutrient-poor environments (Klimeš et al., 1997). Such strategy, besides other advantages, spreads the risk of bud mortality so that we can expect clonal plants to prevail under harsh conditions in particular. Indeed, the proportion of clonal plants increases towards higher latitudes and may reach 90 % in tundra wetlands (Klimeš et al., 1997). However, evidence from true High Arctic, though scarce (Aleksandrova, 1983) indicate that the proportion of clonal plants declines again at this very extreme margin of vascular plant distribution. The elevational pattern of clonal plant distribution seems to be corresponding to this latitudinal pattern although field evidence from extreme elevations is rare as well. In Rupshu region, nearly one third of species are clonal from the lowest elevations up to 5000 m, from where their proportion gradually declines down to zero at the highest elevations (Klimeš, 2003). This is in contrast with earlier reports which suggested increasing proportion of clonal species with deteriorating conditions (reviewed in Klimeš et al., 1997). Klimeš (2003) supposes that the main selective pressure against clonal plants at the highest elevations comes from mechanical stress caused by instability and repeated freezing and melting of substrates which break connections between ramets, affecting their integrity. Moreover, on most places the below-ground organs like rhizomes have problems to penetrate the hard substrate or permafrost. Therefore, non-clonal plants like those with a main tap root or a pleiocorm with only short below-ground branches are favoured under such conditions (Chapter III).

Clonal species consist of two or more physically and physiologically connected units, so called ramets, which can potentially grow independently after a separation. According to the longevity of the functional connection between the ramets, clonal species can be divided into two groups. The first are splitters, e.g. plants with above ground creeping stolons, with limited persistence of the spacers. The other group are integrators, e.g. turf grasses or species with woody below ground stolons, with persistent connection between ramets (van

Groenendael et al., 1996). It is a question in current ecology which habitat conditions favour which strategy of the two, particularly concerning the access to the resources and their utilization. In Rupshu region, integrators were found to reach higher mean and maximum elevations than splitters, and prevail in nutrient-poor environments like semideserts, cold sandy steppes or subnival zone. Integrators also prefer habitats with very stable substrate and dense vegetation cover, which can be found along stream beds. On the other hand, splitters prefer mesic habitats, saline sites and wetlands (Klimeš, 2008). This is consistent with the hypothesis that integrators should be favoured under stressful conditions, like in nutrient poor habitats, whereas splitters are expected to prevail in more favourable environments with more nutrients and moderate climate. However, further analyses revealed that most relationships between clonal integration and environmental factors can be explained by the phylogenetic relation of the species. This means that the related species share these specific traits from their common evolutional history and that the adaptive value of either strategy is probably little (Klimeš et al., 1997; Klimeš, 2008).

In order to say if the patterns mentioned above are general, clonality in plants must be assessed using the same measures. Dicrepancies between results often arise because of different definitions and categorization of clonal plants. We believe that the clonal growth form identification key and the hierarchical nature of the introduced scheme (Chapter III) will certainly allow non-experts to apply these plant functions and make meaningful comparisons between different mountain regions.

Role of cushion plants in high-alpine communities

Cushion plants represent one of the most suitable survival strategies under cold, windy and nutrient-poor conditions. This low and compact growth form thus provides advantage in alpine and arctic zones above all. Cushion plants can be found in these environments in different regions of the world and represent a clear model of evolutionary convergence across phylogenetically unrelated taxa (Körner, 2003; Sklenář, 2009). There are about 338 such species in the world which belong to 78 genera and 34 families.

Cushion plants have long fascinated ecologists (Rauh, 1939) and their ability to ameliorate microsite conditions did not remain unnoticed. Their tightly-knot branches are protected against strong deccicating wind and abrasion. Substrate retains higher moisture under their dense canopy. Litter is not blown away but kept underneath and recycled, thus improving microbial life and nutrient economy. Cushion plants also act as effective heat traps and snow often melts earlier around them. All these effects manifest not only within the cushions but also in their close neighbourhood.

No wonder that other plant species often grow at their edge or even right inside cushions and enjoy these advantages. Indeed, a lot of studies made on association between cushion plants and other plant species have shown this positive association. With practically no exceptions cushion plants were concluded to act as nurse plants facilitating the establishment of other species within their canopy by offering microhabitats that are more favourable for seed germination and seedling recruitment than their surroundings (Cavieres and Badano, 2009). Hence, presence of cushion plants may influence community diversity (Sklenář, 2009). Much of this evidence comes from high-Andean communities (Arredondo-Núňez et al., 2009; a metaanalysis) but also from the Scandes, sub-Antarctic island or Himalaya (Antonsson et al., 2009; Haussmann et al., 2010; Yang et al., 2010). The positive effect of cushion plants was greater with increased environmental severity (Arredondo-Núňez et al., 2009; Badano et al., 2010), i.e. at higher elevations (Cavieres et al., 2002; Fajardo et al., 2008; Antonsson et al., 2009; Yang et al., 2010) and under drier conditions (Cavieres et al., 2006).

Given the harsh conditions cushion plants usually grow in, any amelioration may be of vital importance for the accompanying species and override the adverse effect of competition. Competition and facilitation act at the same time along a gradient of stress but the resulting interaction depends on the severity of conditions (Callaway et al., 2002). This issue is dealt by stress-gradient hypothesis (Callaway et al., 2002) and the greater nursing effect of cushion plants in more severe habitats supports it. Ecological theory on species interactions, however, also suggests that facilitative effects could ultimately decrease under extremely stressfull abiotic conditions (Michalet et al., 2006) or at least not increase constantly (Kaway and Tokeshi, 2007). Extreme cold and aridity are supposed to be the paricular combination of stress factors under which any benefit of facilitation would be overidden (Maestre et al., 2005; 2009) but field evidence was lacking. Such extreme conditions dominate in E Ladakh and also one of the most prominent cushion species of Tibetan Plateau is common here, thus making it an ideal place for testing the stress-gradient hypothesis. We carried out two studies which brought evidence that at the very extreme end of stress gradient facilitative effects need not manifest and that the role of cushion plants as nurse species might not be as general as expected (Chapters IV and V).

Soil phototrophs

Germination of seeds, growth and colonization processes of vascular plants in the subnival and alpine zones are affected by soil conditions and microbial assemblages inhabiting the soil biosphere (Belnap and Harper, 1995; Bliss and

Gold, 1999). In arid areas like Ladakh, there are places with suitable climatic conditions for the development of the microbiotic soil crusts. These crusts result from an intimate association between soil particles and cyanobacteria, algae, microfungi, lichens and bryophytes, which live within, or immediately on the top of the uppermost milimeters of soil (Belnap and Lange, 2001). These microbial assemblages could alter the resource supply by tapping the otherwise unused sources (Johansen, 1993; Heckman et al., 2006). Therefore, they play a key role in the retaining of carbon, nitrogen and liquid water and protect against erosion, which is important for the subsequent plant and animal succession (Belnap and Lange, 2001; Kubečková et al., 2003; Tirkey and Adhikari, 2005; Kaštovská et al., 2005; Schmidt et al., 2008). However, the microbiotic crusts are very fragile and their recovery time is 20 years and more (Belnap and Eldridge, 2001). The crusts can be easily damaged by various disturbances, such as pasturage, tourism or transfers of army vehicles (Belnap, 2002). All the mentioned activities have escalated in the last decades in E Ladakh. When the crusts are damaged, they lose their functionality and the equilibrium of the ecosystem is disrupted (Belnap and Lange, 2001).

Since these microbial soil communities play such an important role in arid ecosystems, it is essential to pay attention to their response to the climatic changes (Evans et al., 2001). There are virtually no data about the soil parameters and about the microbial assemblages from E Ladakh. This is why our team started to focus on this issue, namely the physico-chemical characteristics of soils, species composition and abundance of microorganisms and the interactions between plants and microbial assemblages (Řeháková et al., 2011; Chapter VI).

Coldest places on earth with vascular plants

The subnival zone of the western part of Tibetan Plateau, stretching also to the easternmost Ladakh, is unique for several reasons. I mentioned earlier that thanks to the specific geomorphology and arid climate the habitable zone for vascular plants reaches one of the highest elevations in the world here. As was shown by Körner (2011) and documented also in Chapter VII, it does not necessarily mean that the most elevated places on earth still supporting vascular plant life are also the coldest.

So far, the coldest known place has been discovered at Dom summit in central Swiss Alps at 4505 m and was occupied by *Saxifraga oppositifolia* (Körner, 2011). This plant needed for persistence 66 days of mean temperature >0 °C at the rooting zone (seasonal mean 2.6 °C) and the heat sum of 178 degree days (threshold 0 °C). The plant moreover experienced frost every day including the rooting zone. In Himalaya, there are suitable habitats at higher elevations. These habitats, however, along with sites in High Arctic, were so far documented to be warmer in comparison to that place in Alps (Klimeš and Doležal, 2010; Körner 2011).

Records of plant species above 6000 m are usually obtained accidentally by climbing expeditions and the knowledge about vascular plant life at the highest elevations in the world is thus very limited. Scientific studies on the vegetation and climate from elevations above 5000 m are extremely rare. The current elevational record-holding species is *Saussurea gnaphalodes*, found in 1938 by E. Shipton in the region of Mt. Everest (Miehe, 1991; General Herbarium, British Museum, London). This species is common throughout Tibet and also in our study region. It belongs to a group of several species which occurr above 6100 m in E Ladakh and some of them were record-holders of the highest growing plant in the past. Those other species, e.g. *Ladakiella klimesii* or *Desideria pumila*, may be even hardier and better adapted to extreme conditions, as they seem to be restricted on the highest zone and do not occur much lower than 5600 m, unlike *S. gnaphalodes*. As a matter of fact, *S. gnaphalodes* (Asteraceae) has fruits with effective dispersal mechanism, thus may have better chance to reach a distant suitable microsite. Other species could have flourished there as well but may have simply been unable to reach the site. Limitations posed on species at the upper elevational limit and constraints in the colonizing process are further discussed below (Chapter VII).

As far as I know, no microclimatic measurements were conducted at the highest places of records traded for decades from the Nepalese Himalaya (e.g. *Ermania himalayensis* at 6300 m, *Arenaria bryophylla* at 6200 m or *Stellaria decumbens* at 6100 m; Miehe, 1991) which would show if they were sites with the most extreme (coldest) microclimate which terrestrial vascular plants are able to cope with. We can only speculate what that highest place in 6400 m with *S. gnaphalodes* was like (and if its elevation was measured correctly). It is probably a question of low state of reseach and challenging conditions that colder inhabited microsites have not been found in Himalaya yet. In order to conclude which place on earth is the coldest, yet hosting vascular plants, a systematic search accompanied with proper microclimatic measurements must be conducted. Since elevation per se is a complex factor and explains a small part of the story only, the search for such a cold place should include even low-elevation sites within High Arctic and Antarctic. I sincerely encourage alpine scientists and others for such a hunt.

Objectives and content of the thesis

The region of Ladakh, due to its isolation and difficult accessibility, remains poorly explored in many regards. Modern botanical and ecological knowledge based on systematic field research of the region started emerging only from late 1970s, when Ladakh was opened for foreigners and when it also attracted wider attention of Indian scientific community. Some parts, however, mainly near the politically unstable border with Pakistan and China, are closed up to now.

In this thesis I try to shed light upon the modern scientific discovering of Ladakh and to illustrate the environmental background which makes it so special. Actually, nearly every topic from expert fields of our research team that came to mind was worth looking into and making it clearer. Many issues, so well-known from other parts of the world were simply unknown or unclear here. This matters the more as vegetation in Ladakh reaches global elevational limits and many ecological or physiological patterns and trends may be different to their casual courses at the very extremes (Klimeš, 2003; Körner, 2003; Macek et al., 2012). From this point of view Ladakh represents a kind of "natural experiment", providing very long elevational gradient suitable for comparative functional ecology as well as for testing various hypotheses concerning limitations of vascular plants. Arid climate and extreme elevations are the common factors.

The main objectives of this thesis are: (i) to summarize the main vegetation types in terms of species and growth form composition, (ii) to provide a widely applicable system for classification of clonal growth forms, (iii) to assess species interactions at the extreme end of stress gradient, (iv) to describe soil phototrophs communities along elevational gradient and (v) to characterize the subnival zone with focus on species and microclimate.

Chapter II describes main vegetation types of eastern Ladakh and discusses key environmental factor which influence species and growth form composition.

Chapter III presents a classification of clonal growth forms for vascular plants from eastern Ladakh and assesses the relationship of these forms with multiple environmental gradients.

Chapter IV deals with association between a cushion plant *Thylacospermum caespitosum* and other alpine plants on a site at extreme elevation and provides support for the recently refined hypothesis which predicts a decrease in facilitation under extreme stress.

Chapter V examines association between a cushion plant *Thylacospermum caespitosum* and other alpine plants on elevational gradient and discusses the results in the context of stress-gradient hypothesis.

Chapter VI focuses on community structure of soil phototrophs along environmental gradients and discusses the importance of biological soil crusts in arid environments.

Chapter VII characterizes subnival zone of eastern Ladakh which is one of the regions where vascular plants reach the global elevational limit. It focuses on plant assemblages, distribution of typical species, their growth characteristics and microclimatic conditions. It also discusses constraints and limitations which stand behind the current elevational limit of vascular plants.

Chapter VIII summarises the main results of this thesis.

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CHAPTER II

Vegetation types of East Ladakh: species and growth form composition along main environmental gradients

Dvorský M, Doležal J, de Bello F, Klimešová J, Klimeš L (2011):
Applied Vegetation Science 14: 132-147

Photo on previous page: *Corydalis moorcroftiana* (Fumariaceae), Zanskar region, S Ladakh.

Vegetation types of East Ladakh: species and growth form composition along main environmental gradients

Abstract

The vegetation of high mountain regions in Himalaya remains poorly assessed despite the expected high vulnerability of these ecosystems to global change drivers. What are the main vegetation types in East Ladakh and which environmental factors influence the species composition growth forms distribution? Location of the study was the south-westernmost extension of the Tibetan Plateau, an arid mountainous region, in East Ladakh, Jammu and Kashmir State, India. Species composition was recorded for 369 plots (each 100 m^2). Plots, sampled from 4180 m a.s.l. (bottom of the Indus Valley) up to 6060 m a.s.l. (close to the snowline), covered a wide range of environmental conditions. TWINSPAN clusters discriminated eight ecologically interpretable vegetation types, corresponding to the main habitats in the region: animal resting places, salt marshes, semi-deserts and steppes, shrublands, alpine screes and boulder fields, alpine grasslands, water bodies and subnival zone. The most important environmental factors influencing the species composition were altitude, soil moisture and salinity. Screes and alpine grasslands were found to be the most species-rich. The species were ranged into 20 growth forms with regard to life-formand clonality, with growth forms showing different changes in proportion among vegetation types and along the different environmental gradients. The study summarizes the main vegetation types of East Ladakh in terms of species and growth form compositions. The results can have a heuristic value for designing future monitoring schemes and assess the effects of global change in these diverse, but poorly studied, regions.

Keywords: alpine vegetation, classification, Trans-Himalaya, TWINSPAN

Introduction

On-going climate and land-use changes are increasingly affecting biodiversity in highly constrained environments such as mountain regions (Körner, 2003). However, little is still known about the mechanisms by which different levels of diversity (taxonomic and functional) are distributed in space, what the response of these diversity components in the future could be and how to preserve them (Pauli et al., 2007).

In cold regions of Ladakh (Western Himalaya), more than 1180 vascular plant taxa occur according to the latest systematic floristic survey (Klimeš and Dickoré, 2006). Although this checklist is preliminary and subspecies as well as cultivated and introduced plants are included, this number is much higher than those reported in previously published floras of the region, e.g. 611 species in Kachroo et al. (1977) and 880 species in Kachroo (1993). These traditional sources of information on species richness of Ladakh are still used by various researchers and in different national and international reports. However, this could result in greatly misleading interpretations of the potential conservation value of plant diversity in the region.

In particular, Northwest India is insufficiently explored in terms of floristic diversity and new vascular plant species are still being described (Al-Shehbaz, 2002; Klimeš and Dickoré, 2005; Kirschner et al., 2006; Klimeš and German, 2008). The main reasons for this are probably the difficult accessibility and logistic problems along with political instability of the region. Description of local vegetation types is not only interesting as a scientific objective but also important for conservation. In the light of the changing climate, whose impacts are predicted to be most prominent in high mountains (Stone, 1992) – along with population increase, which puts a yet higher pressure on natural resources – it is necessary to describe more precisely the different existing vegetation types for future monitoring and conservational purposes.

This study aimed at describing the main vegetation types in the poorly known region of East Ladakh in theTrans-Himalaya, India, in terms of species composition and growth form distribution, and relateing these patterns to the changes in environmental factors. The region occupies an extensive plateau with a close relation to Tibet. Owing to its position in the rain-shadow of the Himalaya Range, the whole region receives very low precipitation (<100 mm yr^{-1}), thus forming a high-altitude cold desert (Stewart, 1916–1917; Negi, 1995). The harsh environment of the region imposes great constraints on plants, which must cope with aridity, extreme diurnal temperature fluctuations, strong winds and abrasion, solifluction at the higher altitudes and salinity at the lower altitudes, nutrient-poor soils and a short growing season. Under such conditions, the productivity is generally very low and the vegetation is most often sparse.

Nonetheless, large areas serve as grazing land for yaks, wild sheep and other mammals and host unique flora, with most plants utilized by local people for medicinal purposes (Ballabh and Chaurasia, 2007). Owing to the remoteness and the low population, the human impact has remained limited and confined to traditional nomadic herd-keeping. However, in recent decades both human and livestock populations (mostly sheep, goats and yaks) have increased significantly and overgrazing represents a serious problem in many parts of the region (Bhatnagar et al., 2006). Even if the vegetation structure and composition remain natural under the influence of moderate grazing (Shrestha and Wegge, 2008; Tambe and Rawat, 2009), in the longer term excessive grazing may cause soil degradation, erosion and nutrient-cycle alterations (Holzner and Kriechbaum, 2001).

Methods

Study region

The study region is situated in the eastern part of Ladakh, Jammu and Kashmir State, India (32°14.5´–33°59.7´ N, and 77°47.0´–78°33.4´ E) (Fig. 1). It covers a total area of 6912 km^2 delimited by the Eastern Karakorum Range in the north and the Great Himalaya Range in the south, forming the southwesternmost extension of the Tibetan Plateau; it includes several large brackish lake basins without external drainage. The altitude ranges from 4180 m (bottom of the Indus River Valley) to 6666 m (Lungser Kangri Peak) with vascular plants occurring up to 6060 m (near snowline).

Ladakh lies in the rain-shadow of the Himalaya, which poses a barrier to seasonal monsoon precipitations. The climate is therefore generally arid with mean annual precipitations as low as 50–100 mm (Hartmann, 1983; Wang, 1988). Precipitations are thought to decrease eastward along the Indus Valley from 83 mm at Leh (3514 m, 34°09´ N, 77°34´ E, about 50 km NW of the study region) to 54 mm at Gar in SW Tibet (4232 m, 32°07´ N, 80°04´ E, ca. 160 km SE of the study region) (Miehe, 1990). Evaporation exceeds precipitation at lower and middle elevations. The few climatic data available for higher elevations of East Ladakh suggest a mean annual temperature of around 0 °C. The mean monthly temperature rises above 0 °C from Jun to Aug only and winter temperatures can drop below -30 °C (Klimeš and Doležal, 2010). The substrate ranges from siliceous rocks (Precambrian granites, gneiss) to calcareous and saline sediments.

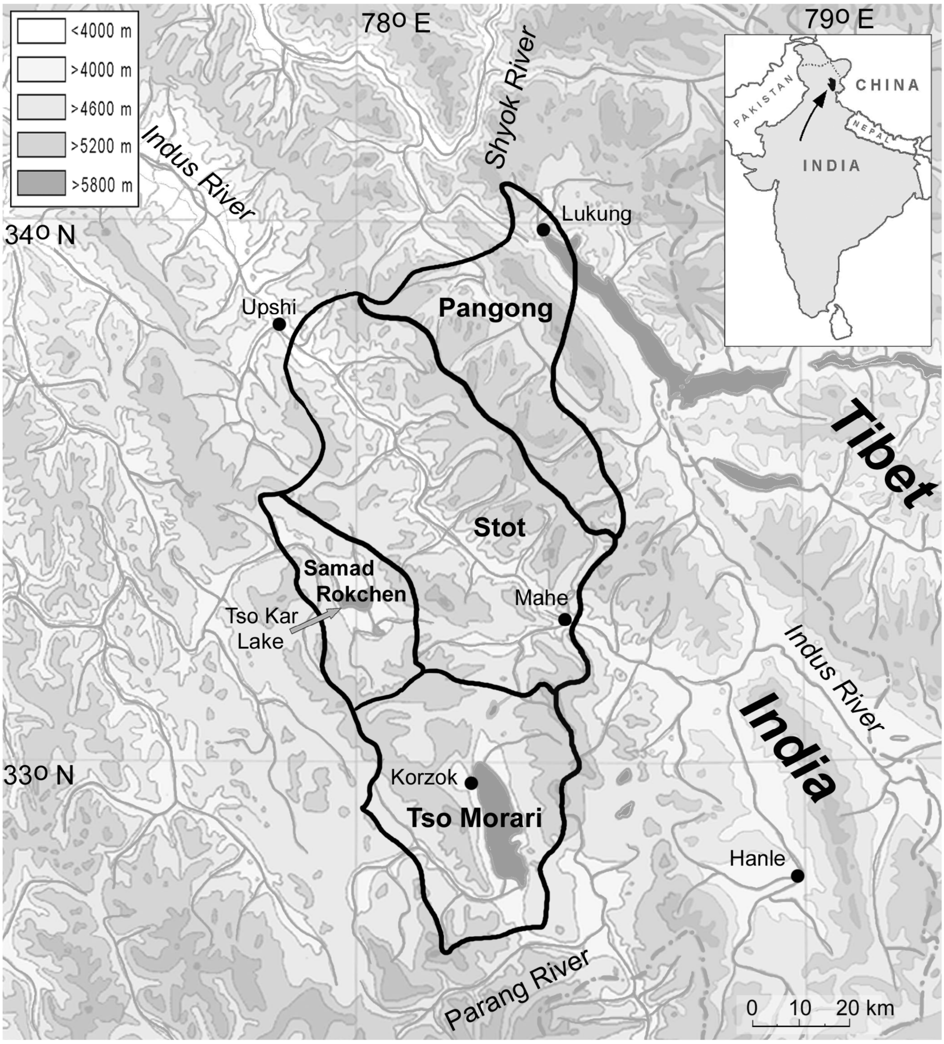

Fig. 1. Study region. The division into four subregions (Pangong, Stot, Samad Rokchen, Tso Morari) is used in Chapter III of this thesis.

Data collection

The field data were collected at nine expeditions lasting 4–7 weeks each, from 1998 to 2003, and in 2005, 2008, 2009. The time of sampling always corresponded with the peak of vegetation season, which lasts from late Jul to mid-Aug in the region. Thus, most likely some early growing ephemeral plants were no longer visible during the sampling. The 369 plots (100 m^2 each) were sampled to cover the physiognomically different vegetation types, across multiple environmental gradients over the region, while avoiding places devoid of vascular plants (very unstable slopes, glaciers, lakes and extremely high elevations [over 6100 m]). Elevation above sea level was estimated with an altimeter (Thommen, Switzerland). In each plot, species composition was recorded and species cover was estimated using a Braun-Blanquet scale. Total percentage vegetation cover was estimated for each plot.

Five main environmental variables were assessed in each plot using the following semi-quantitative scales:
(1) Stability of the substrate: 1 = unstable (screes, dunes, periglacial 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) Nutrient availability: 1 = low (semi-deserts, steppes), 2 = medium, 3 = high (stables, animal resting places);
(5) Soil salinity: 1 = no salt deposits on soil surface, 2 = salt deposits rare, 3 = salts forming a continuous crust.

The percentage composition of the substrate particles in different size classes was estimated directly in the field by the same experienced person. Seven size classes were distinguished according to the diameter of the particles; the size classes of particles smaller than 2 mm in diameter follow USDA Soil Taxonomy system (clay <0.002, silt 0.002–0.05, sand 0.05–2, stones 2–10, stones 10–50, 50–200 and stones bigger than 200 mm) (Baldwin et al., 1938).

For plant species identification, a key to the flora of Ladakh was used (Klimeš, unpublished). Problematic species were collected and determined later with the help of a comparative herbary (deposited in the Institute of Botany, Trebon, Czech Republic). The complete list of species is included in the Supporting Information, Appendix SA1. Abbreviations of species used in graphs have eight characters (formed by the first four letters of the genus name and the first four letters of the species name).

Growth forms

Species were ranged systematically into different growth form according to Klimešová et al. (2011). The methodology applied for this classification is based on an identification key using a series of traits and is similar to methodologies already used for Central European vegetation (Klimeš and Klimešová, 1999). Twenty growth form categories were delimited by combinations of life-history, clonality and mode of clonal growth (type of root system, presence of various structures of vegetative reproduction, presence and length of rhizomes and their position etc.) and named after typical representative species (see Fig. 2 in Chapter III of this thesis, page 58). These growth forms represent an integrated functional classification that enables one to test the response of groups to various environmental variables.

Data analysis

TWINSPAN analysis (Hill and Šmilauer, 2005) was used to generate floristic classification of plots. This cluster analysis starts with primary ordination of sites along the first axis of correspondence analysis (CA) and the plots are then divided into two clusters by splitting the first CA axis near its middle (Hill and Šmilauer, 2005). Plot classification is refined using a discriminant function that emphasizes species preferential to one or the other half of the dichotomy. After three levels of division, the vegetation of the 369 plots was divided into eight ecologically interpretable groups of vegetation types. The vegetation types were named after their diagnostic species (i.e. species with the highest frequency of occurrence).

For each plot the mean values for the following variables were calculated: species richness (number of vascular plants), total vegetation cover, proportions of clonal and non-clonal species, and proportions of the 20 growth forms. The number of species restricted to only one of the vegetation types (unique species) was calculated for each vegetation type. Growth form proportions were assessed on the basis of species presence/absence data in each plot, and then averaged for the vegetation types. Analysis of variance was used to determine differences in environmental characteristics (altitudinal distribution, soil stability, light availability, soil moisture, nutrient availability, salinity) between TWINSPAN vegetation types. To display significant differences in pairwise comparison between TWINSPAN vegetation types in altitudinal distribution and species richness, boxplots with Tukey notches were used. Boxes in which the notches do not overlap indicate significantly different medians. We also used both linear and quadratic regressions to reveal the relationship between the altitude of the plots and their species richness, and performed this for separate vegetation types as well as for all the plots together.

Detrended Correspondence Analysis (DCA) and Canonical Correspondence Analysis (CCA) were used to assess the composition variability across plots and to reveal which environmental factors best relate with the main floristic changes; both methods were conducted using CANOCO (ter Braak and

Šmilauer, 2002). The use of CCA is based on the assumption of unimodal species response (the lengths of the first and second DCA axes were 8.658 and 6.707, respectively, which advocates unimodal methods). Owing to the presence of many rare species with very low cover, the option ‘downweighting of rare species’ was used (ter Braak and Šmilauer, 2002). The constrained and unconstrained methods are complementary: DCA axes correspond to the dominant gradients in species composition, whereas CCA axes correspond to gradients in species composition which best correlate to the measured environmental factors. In CCA, the significant environmental factors obtained by forward selection were used as explanatory variables. We used constrained ordinations to ascribe the explained variability to particular variables using a variance partitioning procedure, in which case the factors not used as predictors were defined as covariables to remove their effects and obtain a net effect of an individual factor (partial CCA; Lepš and Šmilauer, 2003). Monte Carlo permutation tests (999 permutations) were used to assess the significance of the relationships found in multivariate analyses.

Results

A total of 272 vascular plant species were found over the 369 plots, with an average of 10 per plot. These species belong to 43 families (9 in monocots, 33 in dicots plus family Ephedraceae) and 127 genera (24 in monocots, 102 in dicots plus genus *Ephedra*). Eight large families comprised 72.5% of all the species, i.e. Asteraceae (12 genera/44 species), Poaceae (13/34), Brassicaceae (14/23), Fabaceae (6/20), Cyperaceae (4/18), Chenopodiaceae (9/13), Caryophyllaceae (5/12) and Ranunculaceae (5/10). As many as 20 families were represented by only one species. The ratio of monocots to dicots in the region was 1:3.4 (Appendix SA1).

Description of vegetation types

After applying three-level division in the TWINSPAN cluster analysis, we obtained eight groups of relevés corresponding to the main vegetation types. Here, we use the term ‘habitat’ for the type of the environment and its abiotic features, while the term ‘community’ refers to the specific species combination of a vegetation type, which may slightly differ even within one vegetation type over the area of its distribution. The eight distinguished vegetation types differ in terms of altitudinal distribution, species richness and structure (Table 1) as well as in terms of frequent species (Table 2, Appendix SA1).

Table 1. TWINSPAN vegetation types and their main characteristics. The Plot section shows numbers of plots belonging to the respective TWINSPAN vegetation types (n), their proportions in all plots (%) and mean cover values (MC). The Species section shows number of species found within respective TWINSPAN vegetation types (n), proportion of the total of 272 species (%), mean number of species per plot (MS), number of unique species (US; species found within one vegetation type only), proportions of clonal (C) and non-clonal (NC) species. The Altitude section shows mean and maximum values of altitudinal distribution. The Diversity section shows mean values for number of growth forms (GF) and life-forms (LF).

Vegetation type	Plot			Species						Altitude		Diversity	
	n	%	MC	n	%	MS	US	C	NC	mean	max	GF	LF
Puccinellia himalaica-Polygonum sibiricum habitat: salt marshes	92	24,9	57	120	44	8	16	70,9	29,1	4592	5140	4,63	2,77
Elymus jacquemontii-Oxytropis tatarica habitat: shrublands	48	13	20	101	37	10	18	42,3	57,7	4636	5110	4,77	3,33
Potamogeton amblyphyllus-Halerpestes sarmentosa habitat: water bodies	11	3	65	23	9	4	4	89,8	10,2	4695	5110	3,36	2,27
Oxytropis microphylla-Stipa caucasica habitat: semideserts & steppes	83	22,5	21	79	29	10	14	44,6	55,4	4732	5320	4,55	3,51
Kobresia royleana-Kobresia schoenoides habitat: alpine grasslands	54	14,6	71	140	52	15	22	71,5	28,5	4775	5510	6,26	3,04
Chenopodium karoi-Leymus secalinus habitat: animal resting places	9	2,4	56	28	10	6	2	23,6	76,4	4801	5200	3,33	3
Thylacospermum caespitosum-Pleurospermum stellatum habitat: alpine screes	20	5,4	13	85	31	13	11	30,9	69,1	5057	5620	6,15	3,05
Poa attenuata-Potentilla pamirica habitat: subnival zone	52	14,1	37	106	39	11	8	42,1	57,9	5243	5750	5,46	3,19

Puccinellia himalaica-Polygonum sibiricum type (habitat: salt marshes)

This vegetation type includes halophilous communities developed over extensive areas at the bottom of plains, around banks of salty or brackish lakes and near mineral springs. The prevailing substrate was sandy silt with a high salinity level, with salts often forming a continuous crust. Soil fertility level was low. The water level regularly reached the soil surface for a transient period or water covered the surface permanently. The altitudinal optimum was about 4500 m. The vegetation cover was very low (approximately 20%) in places with the highest salinity and highest water level, and increased to 85% in places with more favourable conditions. Species forming this vegetation type were mostly halophilous or salt tolerant hemicryptophytes. Therophytes were represented by few species, the most abundant of which were *Artemisia macrocephala* and *Suaeda olufsenii*.

Elymus jacquemontii-Oxytropis tatarica type (habitat: shrublands)

The characteristic feature of this vegetation type was the dominance of deep-rooted shrubs. The substrate was a mixture of sand and silt with a high proportion of gravel. The nutrient and salinity levels were generally low while water content was moderate to high. The altitudinal distribution varied from 4200 to 5000 m. Vegetation cover was low in most plots, usually not exceeding 30%. The most common and dominant species was the thorny shrub *Caragana versicolor*, being up to 60 cm in height, accompanied by less common shrubs such as *Ephedra gerardiana* and *Krascheninnikovia pungens* (indicating drier substrate) or *Hippophaë tibetana* and *Myricaria germanica* (indicating water surplus). Herbs were represented mostly by perennials and a few annual species, such as *A. macrocephala* and *Polygonum molliaeforme*.

Potamogeton amblyphyllus-Halerpestes sarmentosa type (habitat: water bodies)

This vegetation type included habitats with permanent fresh-water bodies or places with excessive water supply during most of the year. This vegetation was not common in the study region. The substrate consisted of sand mixed with silt and clay and the nutrient level was low to moderate. The vegetation cover was generally high, and in most plots more than 60%. The species of this vegetation type included hydrophytes and hydrophilous species, both annuals and perennials (e.g. *Catabrosa aquatica* and *H. sarmentosa*).

Oxytropis microphylla-Stipa caucasica type (habitat: semideserts and steppes)

This vegetation type occurred over large areas. Semideserts and steppes were the most common, together with salt marshes. The substrate was mostly dry with low nutrient and salinity levels and consisted of sand mixed with bigger stones. The altitudinal optimum range was 4500–4900 m. Insufficient water supply resulted in very low vegetation cover values. Most common species were stress-tolerant hemicryptophytes. Steppe genera such as *Artemisia*, *Oxytropis* and *Stipa* were highly represented. Annuals such as *A. macrocephala*, *Chenopodium karoi*, *Lepidium apetalum* or *Salsola jacquemontii* occurred sporadically and tended to occupy places with better nutrient and/or water availability. *Caragana versicolor* shrubs dominated in some plots although they were not very common as a whole.

Table 2. Synoptic table showing the frequency of the occurrence of the species within the eight vegetation types and the number of plots (of the total of 369 plots) that belong to the respective vegetation types. The vegetation types are sorted from the left to the right according to their average altitudinal distribution. Species are sorted according to their frequency within the respective vegetation types. Species with frequency higher than 30% are shown only. SM, salt marshes; SH, shrublands; WB, water bodies; ST, semideserts and steppes; AG, alpine grasslands; RP, resting places of animals; SC, screes; SU, subnival zone.

Species	**Abbreviation**	**Vegetation type**							
		SM	**SH**	**WB**	**ST**	**AG**	**RP**	**SC**	**SU**
	No. of plots	92	48	11	83	54	9	20	52
Puccinellia himalaica	*PuccHima*	71	6	18	2	30	11	5	4
Polygonum sibiricum	*PolySibi*	41	17	0	4	17	33	0	2
Carex moorcroftii	*CareMoor*	39	0	0	23	9	11	5	29
Elymus jacquemontii	*ElymJacq*	8	69	0	33	13	11	65	29
Oxytropis tatarica	*OxytTata*	3	65	0	49	4	11	60	38
Caragana versicolor	*CaraVers*	1	52	0	10	9	0	10	0
Ephedra gerardiana	*EpheGera*	0	40	0	6	0	0	0	0
Elymus schrenkianus	*ElymSchr*	9	33	0	12	19	22	10	12
Dracocephalum heterophyllum	*DracHete*	1	31	0	27	0	0	15	13
Potamogeton amblyphyllus	*PotaAmbl*	2	0	64	0	2	0	0	0
Halerpestes sarmentosa	*HaleSarm*	46	0	64	0	28	0	0	0
Catabrosa aquatica	*CataAqua*	1	0	45	0	0	0	0	2
Oxytropis microphylla	*OxytMicr*	5	23	0	76	0	0	0	6
Stipa caucasica	*StipCauc*	0	10	0	72	0	0	0	0
Ptilotrichum canescens	*PtilCane*	0	2	0	70	0	11	0	0
Krascheninnikovia pungens	*KrasPung*	2	46	0	58	0	11	0	2
Stipa subsessiliflora	*StipaSubs*	2	23	0	51	0	0	5	15
Chamaerhodos sabulosa	*ChamSabu*	1	0	0	34	2	0	0	4
Kobresia royleana	*KobrRoyl*	18	4	0	0	67	0	20	8
Kobresia schoenoides	*KobrScho*	20	0	0	0	54	0	5	15
Calamagrostis holciformis	*CalaHolc*	32	8	0	1	44	0	15	0
Potentilla multifida	*PoteMult*	20	25	0	0	43	0	5	19
Astragalus strictus	*AstrStri*	11	10	0	2	39	0	5	23
Thalictrum alpinum	*ThalAlpi*	2	0	0	0	37	0	0	0
Lomatogonium carinthiacum	*LomaCari*	18	0	9	1	31	0	0	0
Chenopodium karoi	*ChenKaro*	4	4	0	27	0	56	0	2
Leymus secalinus	*LeymSeca*	23	13	0	25	2	56	0	6
Artemisia macrocephala	*ArteMacr*	22	15	0	8	4	44	0	10
Axyris prostrata	*AxyrPros*	3	4	0	8	0	44	0	2
Microgynoecium tibeticum	*MicrTibe*	2	0	0	0	2	44	0	4
Physochlaina praealta	*PhysPrae*	2	0	0	7	0	33	0	0
Thylacospermum caespitosum	*ThylCaes*	0	0	0	0	7	0	60	48
Pleurospermum stellatum	*PleuStel*	0	4	0	0	9	0	50	2
Rhodiola tibetica	*RhodTibe*	2	4	9	0	24	0	50	8
Marmoritis rotundifolia	*MarmRotu*	0	2	0	0	0	0	45	0
Saussurea bracteata	*SausBrac*	0	4	0	0	11	0	45	15
Poa attennuata	*PoaAtte*	3	33	0	11	31	11	70	83
Potentilla pamirica	*PotePami*	3	10	0	5	33	0	50	63
Saussurea gnaphalodes	*SausGnap*	0	4	0	0	6	0	40	48
Draba altaica	*DrabAlta*	0	0	9	0	9	0	25	44
Arenaria bryophylla	*ArenBryo*	0	4	0	6	4	0	15	38
Carex sagaensis	*CareSaga*	30	2	27	1	22	0	10	35
Stellaria depressa	*StelDepr*	11	2	9	1	22	0	20	31

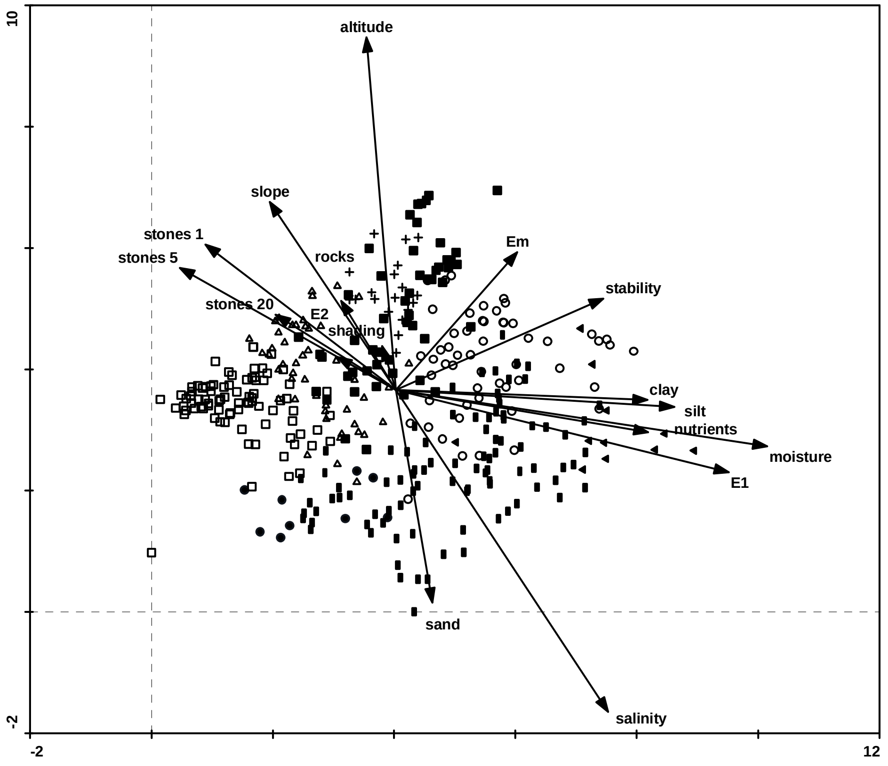

Fig. 2. DCA correlation diagram showing distribution of plots and environmental factors. The first two axes correspond with the most important gradients in the floristic composition of the plots, moisture and altitude in this case. Symbols depict plots belonging to individual vegetation types (+ screes, ■ subnival zone, ○ wet grasslands, ◄ water bodies, ▮ salt marshes, ● animal resting places, □ semi-deserts and steppes, Δ shrublands). Abbreviations: E2, shrub layer cover; E1, herb layer cover; Em, moss and lichen cover; Rocks, percentage cover of stones bigger than 20 cm in diameter; Stones 20, percentage cover of stones in the size class 5–20 cm in diameter; Stones 5, percentage cover of stones 1–5 cm in diameter; Stones 1, percentage cover of stones up to 1 cm in diameter.

Kobresia royleana-Kobresia schoenoides type (habitat: alpine grasslands)

Alpine grasslands occurred mainly along streams and had the greatest species richness in the study region. The substrate consisted of sandy silt and was typically well supplied with water. Nutrient and salinity levels were moderate to low. The altitudinal optimum was between 4600m and 4900 m. The vegetation cover was very high. Large proportion of alpine grasslands was represented by sturdy *K. pygmaea* mats. This vegetation type was dominated by clonal hemicryptophytes; annuals were absent or rare.

Table 3. Correlations of environmental factors with the first two detrended correspondence analysis (DCA) axes, and explained variability and significance levels from the canonical correspondence analysis (CCA) for both marginal effects (variability explained by a given factor without considering other factors) and net effects (variability explained by a given factor after accounting for the effects of other factors (covariables). Significance levels: ***, P<0.001; **, P<0.01; *, P<0.05. Abbreviations: E2, shrub layer cover; E1, herb layer cover; Em, moss and lichen cover; Rocks, percentage cover of stones bigger than 20 cm in diameter; Stones 20, percentage cover of stones in the size class 5–20 cm in diameter; Stones 5, percentage cover of stones 1–5 cm in diameter; Stones 1, percentage cover of stones up to 1 cm in diameter.

Factor	**DCA**	**DCA**	**CCA**	**CCA**
	Correlation with 1st axis	Correlation with 2nd axis	Marginal effect (% explained)	Net effect (% explained)
Stability	0,81	0,16	4.5***	2.0***
Moisture	0,96	-0,1	5.3***	3.0***
Salinity	0,7	-0,64	4.6***	2.1***
Fertility	0,92	-0,04	5.0***	1.8***
Shading	-0,14	0,21	1.6***	1.1***
Altitude	-0,03	0,68	3.1***	1.9***
Slope	-0,25	0,34	1.3***	0.5***
E2	-0,1	0,02	1.7***	1.4***
E1	0,7	-0,17	3.4***	0.6***
Em	0,27	0,24	1.0***	0.5***
Rocks	-0,1	0,17	0.7**	0.5***
Stones 20	-0,24	0,14	1.4***	0.6***
Stones 5	-0,45	0,23	2.1***	0.4*
Stones 1	-0,37	0,25	2.4***	0.5***
Sand	0,07	-0,39	1.1***	0,3
Silt	0,59	-0,04	2.4***	0.4*
Clay	0,54	-0,02	2.1***	0.4*

Chenopodium karoi-Leymus secalinus type (habitat: animal resting places)

This vegetation type of rather limited extent in the study region included gathering places of animals and stables and was typically species poor. The substrate was mesic to dry with a high nutrient content owing to deposition of faeces. The altitudinal optimum was 4600–5000 m. The vegetation cover was low, never exceeding 60% of a plot area. Prevalence of annual species was characteristic (e.g. *Axyris prostrata* and *Microgynoecium tibeticum*).

Thylacospermum caespitosum-Pleurospermum stellatum type (habitat: alpine screes and boulder fields)

This vegetation covered steep slopes with an unstable substrate at high altitudes (4750–5620 m). The substrate consisted of weathered rocks with a high proportion of stones bigger than 20 cm in diameter. Soil moisture was moderate to high, particularly in deeper horizons. The nutrient level was low. Vegetation cover was poorly developed and in most plots varied from 5% to 15%. Dominant species were hemicryptophytes (e.g. *Rhodiola tibetica*). Annuals were absent or rare.

Poa attenuata-Potentilla pamirica type (habitat: subnival zone)

This vegetation type occupied the highest elevations up to the snow-line and included the highest-growing plant communities in the whole Ladakh. Surface stability was moderate, although severely affected by solifluction. Soils were poorly developed and consisted mainly of sand and bigger stones. Water availability was good due to a rather high amount of precipitation, but the nutrient level was low. This vegetation type occurred at altitudes from 4600 to 5700 m, but at extreme altitudes near the snow line only individual plants were discovered. The vegetation cover varied greatly among plots, mostly between 20% and 60%. The majority of species were low-growing hemicryptophytes (e.g. *Draba altaica*, *Saussurea gnaphalodes* or *Saxifraga nanella*).

Species-environment relationships

Detrended correspondence analysis provided the basic outline of the compositional gradients in the data, coherently with the TWINSPAN ordination (Fig. 2). The first two ordination axes explain 9.4% of the variability in species data. A high value of species-environment correlations on the first two axes (r = 0.98 and 0.95) show that the selected environmental factors were determinants of species variation in the data set. They explain up to 22% of the total variability when projected into already determined ordination space (Fig. 2). The second axis is well correlated with environmental data, indicating that there is no single dominant gradient. The projection of environmental variables (Fig. 2) reveals that the first axis is positively correlated with moisture (0.95) and soil fertility (0.92), and with surface stability (0.81) and herb cover (0.69), and negatively correlated with the proportion of bigger stones (>20 cm, -0.23) and gravel (grain size 1–5 cm, -0.44) (Table 3). The second axis is positively correlated with altitude (0.67), and negatively with salinity (-0.72) and percentage of sand (-0.39).

The position of individual species and vegetation types supports this interpretation (Fig. 3). The first floristic gradient describes compositional changes from dry semi-deserts and steppes (with species like *Tanacetum fruticulosum*, *S. caucasica*, *Ptilotrichum canescens*, *Artemisia salsoloides*, *Crepis flexuosa*, *Stipa subsessiliflora*, *Chamaerhodos sabulosa*, etc.) to alpine grasslands andwater bodies (with typical species such as *H. sarmentosa*, *C.*

aquatica, *Eleocharis quinqueflora*, *Juncus thomsonii*, *P. amblyphyllus*, etc.; Fig. 3). The positions of individual clonal-growth and life-form categories (Fig. 4) reveal that the first floristic gradient is positively correlated with the abundance of clonal plants, in particular splitters, plants with long (>10 cm) below-ground hypogeogeic rhizomes (*Poa tibetica* category) prevailing in salt marshes, plants with above-ground stolons (*H. sarmentosa* category) prevailing in water bodies, and plants with short epigeogeic (*Cremantodium ellis*ii category) and hypogeogeic (*Leontopodium ochroleucum* category) rhizomes being more abundant in alpine grasslands. Plants negatively correlated with the first DCA axis are non-clonal plants, chamaephytes, rootsprouters (*P. canescens* category) and perennials with a pleiocorm having short branches (*Arnebia euchroma* category) prevailing in semi-deserts and steppes (Fig. 4).

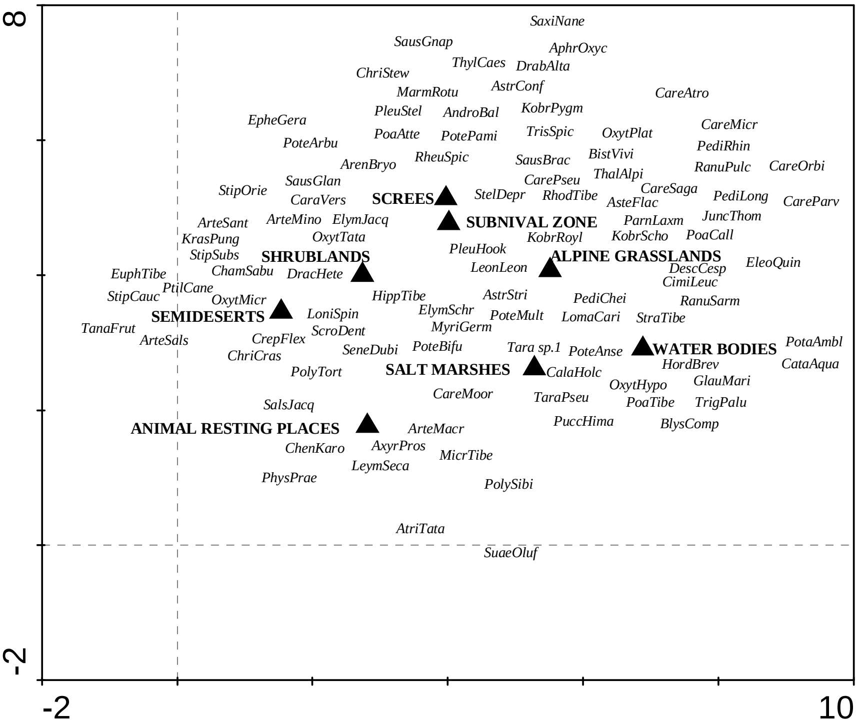

Fig. 3. Indirect detrended correspondence analysis (DCA) showing plant species optimum performances with regard to the main vegetation types in E Ladakh. The first two axes are well correlated with moisture and elevational gradient, respectively. A total of 102 most abundant species are shown. Closed triangles depict the centroids of the vegetation types. For abbreviations of species names see Table 2 and Appendix SA1.

The second floristic gradient is associated with altitudinal changes from salt marshes, forming azonal vegetation on valley bottoms, towards *Caragana* shrublands and alpine screes at higher elevations, and finally to subnival vegetation at the highest positions. The positions of growth and life form categories show that the second floristic gradient is positively correlated with non-clonal plants with a pleiocorm having long branches (*S. gnaphalodes* category), being significantly more abundant in alpine screes, and with compact cushion plants (*T. caespitosum* category) prevailing in the subnival zone (Fig. 4). Annuals and biennials prevailing in animal resting places (higher soil nitrogen and intensity of disturbances) are negatively correlated with the second DCA axis and are also more abundant in salt marshes.

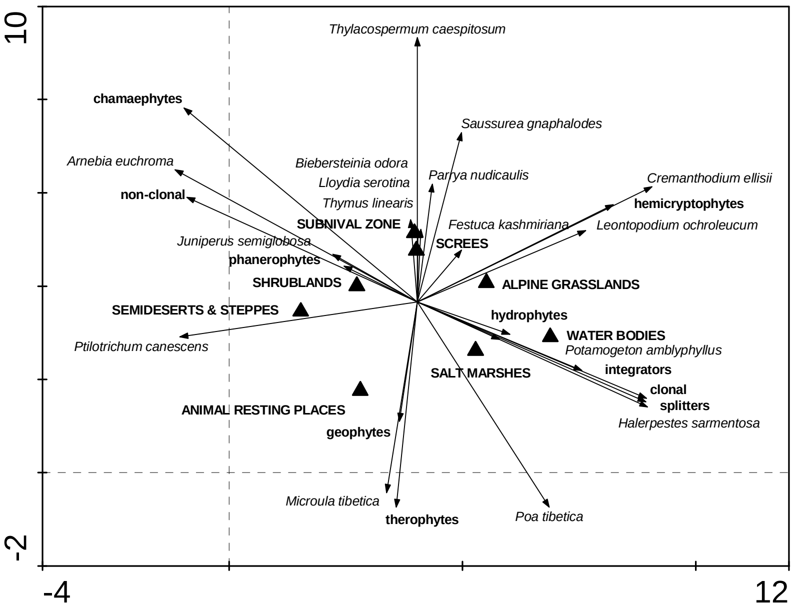

Fig. 4. Ordination diagram showing correlation of distribution of clonal-growth forms, life-form categories and clonal types with respect to the main vegetation types in E Ladakh. Closed triangles depict the centroids of the vegetation types. Names in italic depict the growth forms. Four of the 20 categories are not shown because they were represented by none or one species in the dataset only.

The species richness was also affected by altitude. A regression model for all the plots together showed an unimodal, rather weak, response of the species richness, culminating between 4500 m a.s.l and 5000 m a.s.l. (quadratic

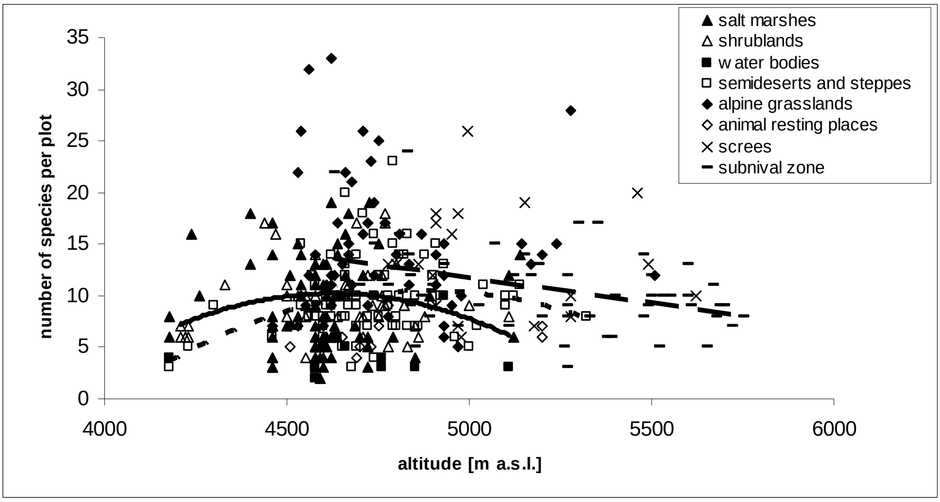

Fig. 5. Species richness and the altitudinal distribution of the plots. Response curves are shown for separate vegetation types in case of a significant trend; shrublands (solid line), semi-deserts and steppes (dotted line), subnival zone (dashed line). A regression model for all the plots together showed an unimodal response of the species richness ($R^2 = 0.03$, $P = 0.004$; curve not shown).

regression, $R^2 = 0.03$, $P = 0.004$; Fig. 5). The regression for plots of separate vegetation types was significant only for semi-deserts and steppes (unimodal response, $R^2 = 0.09$, $P = 0.018$), shrublands (unimodal response, $R^2 = 0.09$, $P = 0.049$) and the subnival zone vegetation (linear response, $R^2 = 0.15$, $P = 0.004$). The altitudinal distribution of the vegetation types and the differences in the species richness are shown in Fig. 6.

Canonical correspondence analysis on relative species cover with habitat variables is summarized in Table 3. In the analysis of all plots, CCA showed that 22.05% of the compositional variability was explained by 16 significant factors selected by forward selection out of 17 explanatory variables tested ($P = 0.001$). The variance partitioning procedure (Table 3) revealed that the soil moisture index is the most important factor influencing species composition, followed by salinity, surface stability and soil fertility indices. Several habitat variables investigated are closely correlated (e.g. percentage of sand and silt). Consequently, after silt was selected, the net effect of sand decreased dramatically. The net effects of all variables investigated were significant with the Monte Carlo permutation test ($P<0.05$), except for sand ($P = 0.082$).

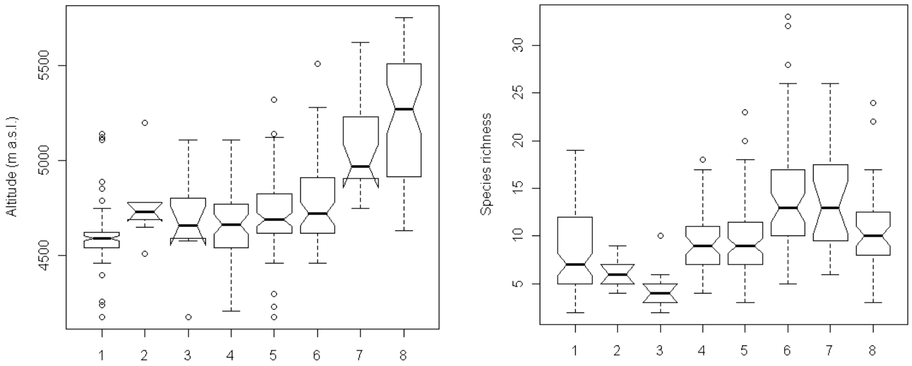

Fig. 6. Boxplots with Tukey notches for pairwise comparisons, showing significant differences between TWINSPAN vegetation types in altitudinal distribution and species richness. (1, salt marshes; 2, animal resting places; 3, fresh-water bodies; 4, shrublands; 5, semi-deserts and steppes; 6, alpine grasslands; 7, alpine screes; 8, subnival zone).

Discussion

As human population and its impact on the environment is on increase even in hardly accessible regions, the knowledge of plant diversity patterns within different vegetation types provides a first sound basis for the potential assessment of conservation priorities and monitoring schemes (Dhar et al., 2000). There are relatively few recent studies dealing directly with the vegetation of the Trans-Himalaya (Kala and Mathur, 2002; Rawat and Adhikari, 2002; 2005a, b; Klimeš, 2003). The most valuable work on the vegetation of Ladakh was provided by Hartmann (1983, 1984, 1987, 1990, 1995, 1997, 1999) who based his geobotanical description of the region on about 280 vegetation records. Kala and Mathur (2002) analysed the floristic composition of Ladakh within preselected landscape types; their results, however, suffer from many taxonomic misinterpretations as the sources of plant identification (Kachroo et al., 1977; Polunin and Stainton, 1984) are incomplete (Klimeš and Dickoré, 2006). At a smaller spatial scale, Rawat and Adhikari (2005a) focused on the vegetation of the Tso Kar basin, a part of our study region, and observed patterns similar to those seen in our study: vegetation types largely follow moisture and altitudinal gradients with plant cover and species richness influenced mostly by microhaitat conditions. To the best of our knowledge, our study is the first comprehensive work that presents extensively the main vegetation types of East Ladakh in the Trans-Himalaya, the underlying environmental gradients that shape them and the sensitivity of growth forms to these environmental factors.

On the basis of differences in floristic composition, eight main vegetation types were delimited. These vegetation types are relatively broad units, which enable the classification of basically every vegetation type occurring across the vast area of East Ladakh into one of eight units. This could be very useful, especially in regions with scarce or incomplete information on vegetation, where such a broad classification could give a first indication of the diversity of the system and the main environmental factors that influence vegetation. Although this classification does not reflect minor differences in ecological conditions among microhabitats, this approach seems appropriate to perform a basic vegetation mapping of rather uniform vegetation of Ladakh.

Salt marshes, semi-deserts and steppes prevail among the vegetation types, providing a first indication of the importance of these communities in the landscape of Ladakh. The desert–steppe communities show similar species composition to neighbouring parts of Karakorum Range (Eberhardt et al., 2007) as well as to other regions of Ladakh. In his works, Hartmann (1995, 1997, 1999) concentrated on this dominant vegetation type (referred to as ‘‘Wü stensteppe’’) and provided a closer sub-classification of different steppe types according to the altitude and composition. Peer et al. (2007) provided a detailed study on *Artemisia* steppes in Hindu Kush in Northern Pakistan; a comparison between our data and that study shows similarities in the structure, but Tibetan floristic elements are represented more in East Ladakh.

Alpine grasslands and the subnival vegetation are common vegetation features of the landscape at the highest elevations. As proposed by Miehe et al. (2008), these specific alpine communities might originate with human pasture activities. *Kobresia pygmaea* is usually the dominant species here, and *Kobresia* mats are generally considered the most characteristic vegetation type over the Tibetan Plateau (Dickoré 1995). Shrublands are less common in the study region; as local people use the shrubs for firewood; this could lead to the reduction of shrubland (Samant et al., 2000). The least common vegetation type is that of fresh-water bodies, which are naturally rare in the study region, while the vegetation of animal resting places is expected to increase with the rising number of domestic animals.

Although strongly overlapping, there are significant differences in the mean values of the altitudinal ranges of the vegetation types. The lowest elevations are occupied by salt marshes, fresh-water bodies, shrublands, semi-deserts and steppes, all of them occurring on valley bottoms and around salty lakes (Tso Moriri, Tso Kar, Pangong). Both fresh-water bodies and salt marshes are limited to specific sites, but salt marshes are much more common and occur up to 5140 m a.s.l. Semi-deserts and steppes as zonal formations are the most common vegetation types in the whole region, dominating vast plains and gentle slopes of high-altitude plateaus continually up to 5320 m a.s.l. The vegetation type of animal resting places is scattered along the elevation gradient quite haphazardly because its distribution is partly of human origin. Alpine grasslands often follow streams along their entire length in a wide altitudinal range from 4460 to 5510 m a.s.l. The distribution centre of screes and of subnival vegetation is mostly above 5000 m a.s.l. with exceptions at lower sites, such as narrow gorges of subalpine streams (screes) and northern or exposed windward slopes (subnival vegetation). At about 6000 m a.s.l., only individual plants are observed, scattered at microsites that provide at least a minimal shelter against severe conditions (e.g. bigger stones). The highest growing vascular plant (*Saussurea hypsipeta*) was found at 6060 m a.s.l., which is the highest occurrence of a vascular plant known from the region.

Regarding plant diversity, alpine grasslands in East Ladakh can be characterized as the most species-rich vegetation type, moreover harbouring the highest number of unique species. This fact was also documented by Rawat and Adhikari (2005a), who described moist meadows to have the greatest species richness in the region. Hartmann (1968) in his study from Biafo Glacier region in the neighbouring Karakorum Range described a similar vegetation type of alpine grasslands (‘‘alpine Wiesen’’) with dominant *Carex* and *Kobresia* species and also considered it to be the most species-rich community. Alpine grasslands very likely experience the greatest grazing pressure in the whole region, so the dominance of a few clonal species of graminoids is in accord with their grazing history (Bock et al., 1995). However, the processes connected with grazing (nutrient deposition, trampling creating new microsites for seedling establishment and reducing the biomass of dominants provided by animals) create suitable conditions for species coexistence (Bakker, 1989; Olff and Ritchie, 1998). In comparison, alpine screes harbour a much lower number of species, but this vegetation type has a comparable mean number of species per plot and the greatest diversity of growth forms. An explanation for the greater species richness of screes might be the fact that this habitat is apparently not as hostile as it seems; an accumulation of fine substrates among big stones and a specific microclimate (higher humidity, less extreme temperature fluctuations, etc.) likely favour pedogenetic processes in deeper layers so that species with a long taproot can profit from this habitat (Körner, 2003). Animal resting places and freshwater bodies were poorest in species (see also Kala and Mathur, 2002; Rawat and Adhikari, 2005a), which partly results from the limited extent of these habitats in the study region and the fact that high disturbance regime is likely to reduce diversity by increasing plant mortality (Bakker, 1989; Olff and Ritchie, 1998).

Across all vegetation types, the greatest species richness was observed between 4500 and 5000 m a.s.l., with a gradual decline towards both lower and higher altitudes. A similar trend with the unimodal distribution of the species richness was shown for the vegetation of semideserts and steppes, indicating that this zonal vegetation has its optimum around 4800–5000 m a.s.l. in the region. According to our preliminary results, this can be ascribed to water limitation at lower elevations and temperature limitation at higher elevations, respectively. Subnival vegetation is the only type showing significant linear decline of species richness, suggesting that the subnival zone is enriched by other species at lower altitudes, whereas higher up it consists of fewer subnival specialists.

Life-forms and clonality in East Ladakh were already partially studied by Klimeš (2003; see also Hartmann, 1995) but, here, we expand this description and combine it with a more comprehensive classification scheme and vegetation description. The above-mentioned authors concluded that the life-form spectrum is closer to desert-steppes and steppes (e.g. a high proportion of hemicryptophytes, a high number of turf grasses and a low proportion of therophytes) rather than to deserts or cold deserts, as the vegetation of Ladakh is often referred to by local authors (Negi, 1995). Clonal species prevailed in habitat types with good water availability and average nutrient status: in salt marshes an of average 70.9% of the species present were clonal, in water bodies 89.8%, and alpine grasslands 71.5%. Of the clonal species, splitters prevailed in water bodies only (67.9% of all species), while integrators were dominant in alpine grasslands (68.4%) and salt marshes (60.4%). Accordingly, Rawat and Adhikari (2005a) found the communities of wet meadows to have the highest density of stems because of the dominance of clonal species forming tillers and dense tussocks. Non-clonal species were prevalent in the rest of the vegetation types with the highest representation in animal resting places (76.4%) and screes (69.1%). The proportion of non-clonal species increases with altitude, as was already reported by Klimeš (2003). The large number of non-clonal species in animal resting places results from a large number of annual species, attaining on average 55.5% of all species present. On screes, in addition to the majority of species being non-clonal, practically all the clonal species belonged to the integrator type (94.2% of clonal species). This suggests the advantage of a compact growth in conditions with frequent disturbances. The advantage is also apparent for plants with a main taproot, which is located in suitable deeper layers of scree and which enables regeneration of branches lost in a disturbance in upper layers. Both solifluction with its daily thaw-freeze cycles at high altitudes and movements of stones and boulders in screes damage the connections between ramets (Klimeš, 2003). Thus, nutrients and energy invested into ramet production and connection maintenance are lost, and this leads to a selection against such clonal types (Körner, 2003). Finally, it is rather difficult for rhizomes to penetrate hard substrates, which frequently occur in East Ladakh, so that compact growth forms are favoured here (Klimešová et al., 2011).

In conclusion, we proposed a classification system of the main vegetation types in East Ladakh, and we related the distributional patterns of these types to the main environmental factors. Such a classification system could be useful for a large-scale vegetation mapping of the region as well as a prerequisite for designing conservational practices.

Supporting information

Additional supporting information may be found in the online version of this article:

Appendix SA1 Checklist of species recorded in the plots and their frequencies within the eight vegetation types. The species abbreviations used in graphs are formed from the first four letters both from genus and species name. Abbreviations: SM, salt marshes; SH, shrublands; WB, water bodies; ST, semi-deserts and steppes; AG, alpine grasslands; RP, resting places of animals; SC, screes; SU, subnival zone.

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CHAPTER III

Clonal growth forms in eastern Ladakh, Western Himalaya: classification and habitat preferences

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Photo on previous page: *Cremanthodium ellisii* (Asteraceae) in Zanskar region, S Ladakh.

Clonal growth forms in eastern Ladakh, Western Himalaya: classification and habitat preferences

Abstract

Earlier observations that plant clonality, i.e., production of potentially independent offspring by vegetative growth, increase in importance in cold climates such as in arctic and alpine regions, have been recently questioned. However, lack of data obtained using a comparable methodology throughout different regions limit such comparisons. Here we present a classification of clonal growth forms for vascular plants from East Ladakh (an arid mountain range in NW Himalaya, India), and assess the relationship of these forms with multiple environmental gradients. Based on field assessment of clonality in 540 species we distinguished 20 growth forms, which were then grouped into four broader space occupancy strategies. Occurrence in communities and relationship with environmental characteristics and altitude were analyzed using multivariate methods. The most abundant growth form was represented by non-clonal perennial species with a pleiocorm having short branches, prevailing in steppes, *Caragana* shrubs and screes. The most abundant clonal species were those with very short epigeogenous rhizomes, such as turf graminoids prevailing in wet *Kobresia* grasslands. Two principal environmental gradients, together with several abiotic variables, affected space occupancy strategies: moisture and altitude. Non-spreading integrators prevailed on shaded rocky slopes, non-spreading splitters in wet grasslands and spreading splitters at the wettest sites. Spreading integrators were the least frequent strategy predominantly occurring at the most elevated sites. Because relevance of clonality decreased with altitude and different communities host different sets of clonal growth strategies, comparison with other cold climate regions should take multiple environmental gradients into account.

Keywords: altitude, classification, clonal growth form, clonal space occupancy, strategies, habitat types, Ladakh

Plant nomenclature according to Klimeš and Dickoré (2006)

Introduction

The ability of plants to grow clonally, i.e., to produce potentially independent offspring, is considered to be important especially in stressful environments such as cold regions of high mountains and the Arctic (Bliss, 1971; Billings, 1974; Callaghan et al., 1992; Jónsdóttir et al., 1996; Klimeš et al., 1997; Körner, 1999a; Evette et al., 2009). However, preliminary analyses show that this relationship is not as universal as expected (Klimeš, 2003; Klimeš and Doležal, 2010). For further understanding of the relevance of clonality in cold regions, comparable data obtained with standardized methodologies are needed. This requires the use of a general classification scheme of clonal versus non-clonal growth forms.

The classification of plant species into easily distinguishable growth forms has a long tradition in botany, dating back to von Humboldt (1806) and his attempt to describe physiognomic plant types in taxonomically unknown vegetation of South America. The idea of a common ecological function of similar gross morphologies was proposed by Grisebach (1872), Drude (1887), Raunkiaer (1907), and Warming (1923) in their classifications focusing on different environmental factors, e.g., climate or soil. Although other growth form classifications have been developed (Du Rietz, 1931; Gimingham, 1951; Hejný, 1960; Lukasiewicz, 1962; Den Hartog and Segal, 1964; Serebrjakov, 1964; Parsons, 1976; Hallé et al., 1978; Barkman, 1988; Halloy, 1990; Kästner and Karrer, 1995; von Lampe, 1999; Krumbiegel, 1998; 1999), few are generally, or at least repeatedly, employed in ecological studies. Despite these there are indeed different ecomorphological plant traits that are widely used in ecological studies to understand the functionality of plants and ecosystems. These are, e.g., the life forms delimited by Raunkiaer according to the highest buds on plants surviving unfavourable seasons (Raunkiaer, 1910), phalanx versus guerrilla growth forms distinguishing two contrasting space occupancy strategies of clonal plants (Lovett Doust, 1981), and splitters versus integrators distinguishing strategies of clonal plants either reallocating or conserving resources (Jónsdóttir and Watson, 1997). However, other ecomorphological traits have a potential to become similarly important, namely clonal growth organs (e.g., stolons, rhizomes or roots), the functionality of which is often studied in comparative experimental studies (see van Groenendael and de Kroon, 1990; de Kroon and van Groenendael, 1997), although their importance at the community level remains rarely investigated (but see Tamm et al., 2002; Halassy et al., 2005, Sosnová et al., 2010).

Although sometimes difficult to standardize across different taxa, a limited set of morphological characters relevant to clonality can be established and can overcome the contingency of the growth form approach of classifications in particular studies and ecosystems (Klimeš et al., 1997; Klimeš and Klimešová, 1999; 2000; 2005; Klimešová and Klimeš, 2008). In this approach it is first essential to identify which organ bears buds for shoot iteration (sensu Hallé et al., 1978), which organ provides connections between ramets, and where these organs are placed. Then it is necessary to assess whether adventitious roots are formed and whether specialized storage organs are present. These principles were used for the classification of clonal growth organs for plants of Central Europe with the notion that a similar classification is applicable with some minor ad-hoc modifications in other regions (Klimešová and de Bello, 2009).

In this study we develop and apply such an ecomorphological classification for 540 species from the high mountain range in Eastern Ladakh, i.e., in the dry West Himalaya, to test the suitability of principles of the clonal growth organ classification in a floristic region of high altitude. We were aware of other growth form classifications aiming to describe clonal propagation in the alpine zone (Hess, 1909; Hartmann, 1957; Rauh, 1987; Halloy, 1990; Körner, 1999a), however we considered the effort to include both the above- and below-ground architecture of plants proposed in most of them as too complicated for the purpose of describing clonal morphological traits and assessing the relevance of space occupancy strategies in cold regions. We are aware, however, that classifications resulting in 20 categories can limit generalizations across regions. Therefore, we grouped the morphological categories according to their capacity to spread (spreading and non-spreading) and the longevity of their connection (integrator or splitter). The resulting four basic clonal strategies in space occupancy are delimited according to the spectrum of traits found in the region.

Eastern Ladakh has a harsh climate, where plants are subjected to multiple stresses such as low precipitation (50–100 mm per year), extreme diurnal temperature fluctuations, strong winds, solifluction at higher and salinity at lower altitudes (Stewart, 1916–1917). Habitat types and plant communities are relatively sharply delimited by differences in abiotic factors (mostly related to altitude, substrate and moisture availability) and plant species composition (Dvorský et al., 2011). Environmental selection pressure is strong and is presumably not masked by biotic interactions (Körner, 1999b). Due to the remoteness of E Ladakh, the local flora is little affected by plant invasions even though human activities, such as grazing of domestic animals and collection of fire wood, have influenced the vegetation substantially despite low human population.

Ladakh in the NW Himalaya is often termed a “cold desert” (Chowdhery and Rao, 1990). Although the flora is species-poor (Dickoré and Miehe, 2002), it includes almost the entire spectrum of life and growth forms (Klimeš, 2003) and most of the important families of the Holarctic flora, except for exclusively woody taxa. Growth form spectra and their altitudinal distribution in the study region were determined in four broad geographical sub-regions, between 750 and 3100 km^2 in size. The study region comprises one of the most prominent altitudinal gradients inhabited by vascular plants in the world, ranging from 3550 to 6030 m above sea level.

Our study had three aims: i) to classify clonal growth forms of the study region on a morphological basis and to combine them into four basic clonal strategies of space occupancy, ii) to assess clonal growth spectra of plant communities, iii) to evaluate their distribution along environmental gradients, in particular the elevation gradient, i.e., to test whether morphologically delimited clonal growth forms have a functional relevancy. To correlate clonal growth form spectra and environmental conditions, both at the regional flora and the plant community levels, we used two different approaches, i) cross-species analysis and ii) relative species abundance within a vegetation type. Studies of trait responses based on equal importance of species may yield results different from those considering species abundance. Because growth form traits are correlated with commonness/rarity within a stand (Pakeman and Quested, 2007), trait–habitat relationships based only on species presence/absence data tend to overestimate rare forms and underestimate common forms (Grime et al., 1988; Grime, 2006; Pakeman et al., 2008).

Methods

Study region

The study region comprises the eastern part of Ladakh (Jammu and Kashmir State, India), situated between 32°41.5′–33°59.7′ N and 77°47.0′–78°33.4′ E, and covering 6912 km^2. Eastern Ladakh extends between altitudes of 3550 and 6666 m (Lungser Kangri Peak), along both sides of the Upper Indus River between the Eastern Karakorum range in the north and the main range of the Western Himalaya to the south, and includes the south-western most extension of the Tibetan Plateau with three high-altitude internal drainage basins. The study region can be further divided into four districts: Pangong, Stot, Samad Rokchen, and Tso Moriri (Klimeš, 2008), 760 to 3080 km^2 in size (see Fig. 1 in Chapter II of this thesis, page 24). Due to the large differences in species composition these can be considered as distinct bioregions; only 197 species out of the 540 found in Eastern Ladakh were found in all four regions (Table 1). The whole region is generally arid with vascular plants growing from 3550 m (bottom of Indus Valley) up to 6060 m a.s.l. (snowline).

Eastern Ladakh is rarely affected by monsoonal precipitation, which fails to cross the main Himalayan range (Bhattacharyya, 1989). Mean annual precipitation decreases eastward along the Indus Valley from 115 mm at Leh (3514 m, 34°09′ N, 77°34′ E, about 50 km NW of the study region) to 54 mm at Gar in SW Tibet (4232 m, 32°07′ N, 80°04′ E, ca. 160 km SE of the study region). Evaporation exceeds precipitation at lower and middle elevations. The few climatological data available for higher elevations of Ladakh suggest that the mean annual temperature is around -8.2 °C; the mean monthly temperature rises above 0 °C from June to August only (Miehe et al., 2001). Climatic measurements in the Tso Moriri region in 2003 showed that the soil temperature at the upper distributional limit of vascular plants at 6030 m a.s.l. remained above zero only between mid-July and the beginning of September (Klimeš and Doležal, 2010). The substrate ranges from siliceous rocks (Precambrian granites, Tso Moriri gneiss) to calcareous or saline sediments.

A general description of the vegetation of this region is provided by Hartmann (1983; 1984; 1987; 1990; 1995; 1997; 1999), Rawat and Adhikari (2005), Kala and Mathur (2002), and Klimeš (2003). Due to the low precipitation, steppe vegetation dominated by several *Stipa* and *Artemisia* species prevails in the whole region up to 5300 m in altitude (Fig. 1). Other frequent species include *Oxytropis microphylla*, *Tanacetum fruticulosum* and *Ptilotrichum canescens*. Only small areas at the relatively lowest elevations of the Indus Valley are occupied by semi-desert. A narrow belt of alpine grasslands occurs along water streams running from melting glaciers, where *Kobresia pygmaea* and *Carex pseudofoetida* usually dominate. Alpine and subnival vegetation, characterized by *Astragalus confertus*, *Thylacospermum caespitosum* and *Potentilla pamirica* has developed above 5300 to 5700 m. Vascular plants occur regularly up to 5900 m, locally up to 6060 m above sea level. Salt marshes and wetlands, which occupy relatively large areas of flat and broad valley bottoms in the whole region, represent extrazonal vegetation (Fig. 1). Synanthropic vegetation is developed on eutrophicated soil in the vicinity of stables of domestic animals and near villages up to 5400 m. Steppes and alpine grasslands are grazed by sheep, goats and yaks up to 5600 m (Jina, 1995; Holzner and Kriechbaum, 1998).

Growth form categorization

The methodology to categorize species into different growth forms is derived from the approach used to categorize the Central European flora as proposed by Klimeš et al. (1997). Although the basic idea of this classification had already been implicitly used to assess the altitudinal distribution of life forms and clonality in the region (Klimeš, 2003) and to test the hypothesis whether split or

Table 1. Number and percentage of species recorded in the four study regions, categorized into 20 growth forms according to life-form and clonality (growth form types), and their mean and maximum elevations. The individual growth forms are named after representative species. Identical superscripts within rows denote mean and maximum values not different at significance level P = 0.05 (Tukey HSD).

Growth form	All		Pangong				Stot				Samad Rokchen				Tso Morari					
	n	%	n	%	mean	max.	n	%	mean	max.	n	%	mean	max.	n	%	mean	max.	$F_{(mean)}$	$F_{(max)}$
1 – *Microula*	150	27.5	65	22.7	4612^{a}	4785^{a}	133	27.1	4315^{b}	4658^{a}	66	23.3	4857^{c}	5036^{b}	84	25.0	4779^{b}	4973^{a}	59.1***	13.9***
2 – *Arnebia*	125	23	66	23.1	4808^{a}	5044^{a}	113	23.1	4644^{b}	5042^{a}	65	23.0	4990^{c}	5227^{b}	76	22.6	4954^{c}	5241^{b}	16.6***	4.62**
3 – *Saussurea*	40	7.3	23	8.0	5047^{a}	5288^{a}	38	7.8	4833^{b}	5238^{a}	26	9.2	5138^{a}	5419^{a}	27	8.0	5107^{a}	5470^{a}	4.05**	1.36
4 – *Thylacospermum*	14	2.6	10	3.5	5273	5547^{a}	11	2.2	5428	5789^{b}	10	3.5	5400	5671^{c}	9	2.7	5527	5933^{d}	2.13	14.7***
5 – *Parrya*	2	0.4	2	0.7	5030	5235	2	0.4	5168	5665	2	0.7	5261	5475	1	0.3	5605	5940	1.13	0.98
6 – *Ptilorichum*	10	1.8	5	1.7	4494^{a}	4668	9	1.8	4212^{b}	4600	4	1.4	4800^{a}	5020	7	2.1	4808^{a}	5051	8.45***	1.91
7 – *Halerpestes*	10	1.8	8	2.8	4750	5030	9	1.8	4557	5038	8	2.8	4770	4944	10	3.0	4813	4986	1.35	0.12
8 – *Thymus*	5	0.9	1	0.3	4297	4297	3	0.6	4368	4368	2	0.7	4558	4565	1	0.3	4855	4880	0.48	0.50
9 – *Festuca*	70	13	41	14.3	4782^{a}	5033	61	12.4	4658^{b}	5077	38	13.4	4908^{a}	5128	45	13.4	4910^{a}	5153	7.31***	0.61
10 – *Cremanthodium*	44	8.1	23	8.0	4868	5070	41	8.4	4827	5242	24	8.5	5034	5285	31	9.2	4937	5218	1.90	1.16
11 – *Biebersteinia*	2	0.4	2	0.7	5160	5270	2	0.4	5123	5530	2	0.7	5218	5435	1	0.3	4903	5170	0.35	0.33
12 – *Leontopodium*	7	1.3	4	1.4	4856	5087	7	1.4	4592	4844	3	1.1	4880	5140	4	1.2	4753	4963	0.61	0.29
13 – *Poa*	33	6	23	8.0	4694^{a}	4976	30	6.1	4497^{b}	4989	23	8.1	4806^{ac}	5038	25	7.4	4872^{c}	5164	10.76***	1.00
14 – *Potamogeton filiformis*	3	0.5	2	0.7	4504^{a}	4710	3	0.6	4342^{b}	4790	3	1.1	4683^{c}	4795	3	0.9	4653^{ac}	4763	13.15**	0.14
15 – *Lloydia*	1	0.2	1	0.3	5335	5400	1	0.2	5100	5500	1	0.4	5100	5300	1	0.3	5325	5350	-	-
16 – *Bistorta*	1	0.2	1	0.3	4625	4950	1	0.2	4725	5250	1	0.4	5030	5200	1	0.3	4735	4930	-	-
17 – *Saxifraga*	1	0.2	1	0.3	5385	5570	1	0.2	5385	5900	1	0.4	5255	5650	1	0.3	5270	5890	-	-
18 – *Potamogeton amblyphyllus*	4	0.7	1	0.3	4770	4770	2	0.4	4303	4560	1	0.4	4670	4670	0	0.0	-	-	-	-
19 – *Potamogeton perfoliatus*			0	0.0	-	-	0	0.0	-	-	-	0.0	-	-	0	0.0	-	-	-	-
20 – *Juniperus*	24	4.4	7	2.4	4434^{a}	4504^{a}	23	4.7	4079^{b}	4381^{a}	3	1.1	4823^{c}	5017^{ac}	9	2.7	4739^{c}	4929^{b}	23.29***	5.76**
Total	546	100	286	100	4778	4998	490	100	4560	4945	283	100	4951	5172	336	100	4909	5166		

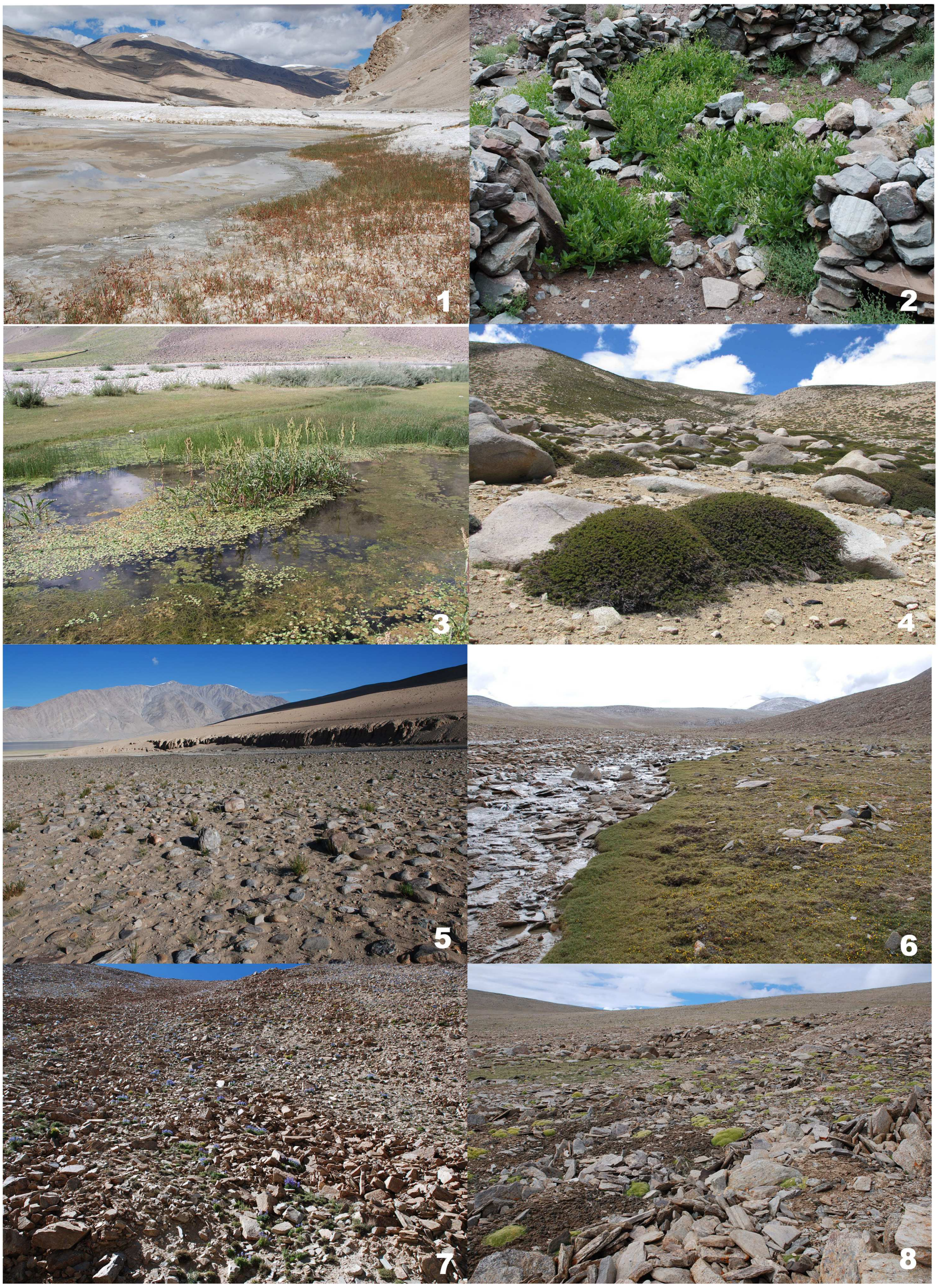

Fig. 1. Eight major vegetation types in East Ladakh. 1 – salt marshes; 2 – animal resting places; 3 – water bodies; 4 – shrubs; 5 – steppes; 6 – wet meadows; 7 – screes; 8 – subnival zone.

integrated clones prevail in the harsh conditions of the region (Klimeš, 2008), a general and more detailed description of this classification is necessary to enable comparative studies in other regions.

The approach is based on the assessment of a set of morphological plant traits related to clonal growth reproduction, i.e., persistence at a spot, lateral spread and clonal propagation (Fig. 2). These traits are hierarchically organized to form a hierarchical trait-based identification key (Appendix 1), which can be applied and compared in other regions of the world by experts and, after some basic training, also by nonexperts. The advantage of producing many distinguishable groups with this approach is that i) this can be generalized across regions and ii) possible subsequent grouping into a more reduced number of groups can be based on the same hierarchical structure in different regions, and therefore allows for meaningful comparisons.

The classification is based on the following questions: which organ bears buds for iteration of shoots, which organ provides connections between offspring shoots, if this organ is short or long, if a plant is able to form adventitious roots, and if there are special storage organs. There are three exceptions: annuals and biennials, woody plants and cushion plants are assessed according to their whole morphology. Short-lived monocarpic plants are non-clonal, trees and big shrubs are very rare in the region and cushion plants are a traditionally distinguished growth form in the alpine regions. Traits not taken into account in the classification, for example, plant size, number and spatial distribution of shoots, distribution of leaves along a shoot, and lifespan of leaves. We also avoided a delimitation of categories primarily according to taxonomy. Non-clonal plants are represented by a particularly heterogegeous group, including short-lived monocarpic species (annuals and biennials) and trees, cushion plants with a tap-root and perennial herbs lacking adventive roots and buds. Although their growth forms differ substantially, they form homogeneous group from point of view of clonality: they are not able to form offsprings by vegetative growth.

The resulting 20 categories (with the exclusion of annual plants) were further grouped into clonal strategies of space occupancy (Fig. 3) according to two characteristics: 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 root). Although the division into integrators and splitters was used in a preceding work from this region (Klimeš, 2008), here we simplified the scheme by lumping together all plants belonging to a certain clonal growth form category and including non-clonal perennials with a perennial main root, as this category is the most plastic and potential clonality may occur in plants occurring in wetter habitats or after disturbance of below-ground plants.

Plants for morphological description, optimally several individuals of each species, were excavated with below-ground organs and dried between paper sheets for future comparison and identification. Plant material is stored in two herbaria, PRA (Institute of Botany, Academy of Sciences of the Czech Republic) and BSD (Botanical Survey of India, Northern Circle Herbarium, Dehradun).

Floristic and vegetation data

The field data were collected on eight expeditions lasting four to seven weeks each, from 1998 to 2003, in 2005 and in 2008. 540 species were recorded at a total of 1282 localities (each approx. 1 ha in size). The localities were non-randomly distributed, as large areas are devoid of vascular plants (very unstable slopes, glaciers, lakes, extremely high elevations). Attempts were made to cover all elevations and habitats in all parts of the delimited regions. Depending on habitat diversity, accessibility, species richness and elevation, plants at each locality were searched for about 30 minutes to one day. The final number of records was 27,018 and these were used to calculate the mean and maximum elevation of each species in the four study regions of E Ladakh. The elevation (in meters above sea level) was estimated with an altimeter (Thommen, Switzerland). The number of vascular plant species recorded in the four study regions ranged from 279 to 486, with a total of 540 (Table 1).

Habitat preferences of individual growth form categories were analyzed using vegetation records from 369 plots (each 100 m^2), sampled in a stratified design to cover visually assessed differences in vegetation types over the whole region. The samples were non-randomly distributed, as large areas are devoid of vascular plants. In each plot, species composition and altitude were recorded and cover of individual species estimated on a semi-quantitative scale. Five environmental variables, considered to be the most important for habitat differentiation, were assessed in each plot using the following scales:

(1) stability of the soil/substrate surface (the inverse of disturbance frequency): 1 – unstable (screes, 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) nutrient availability: 1 – low (semi-deserts, steppes), 2 – medium, 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.

Data analysis

When a species was classified in several categories of clonal growth form its occurrence in these categories was expressed proportionally to the number of categories, so that the final value per species was 1.

Whether the mean and maximum elevations of individual growth form categories differ between the four study regions was tested using ANOVA followed by Tukey's pairwise comparisons (Table 1).

The habitat preferences of individual growth forms were assessed using both unweighted data and data weighted by abundance. We first characterized the samples by the averages of trait values of the constituent species (as traits are categorical variables, the value represents the relative proportion of a species belonging to the given growth form, weighted or not by their cover), and then we compared the growth form spectra between major vegetation types derived from Two-way Indicator Species Analysis (TWINSPAN). This cluster analysis starts with primary ordination of sites along the first axis of correspondence analysis (CA; see Hill and Šmilauer 2005 for details) and the sites are then divided into two clusters by splitting the first CA axis near its middle. Site classification is refined using a discriminant function that emphasizes species preferential to one or the other half of the dichotomy. We repeated this process to classify the vegetation of the 369 plots into eight ecologically interpretable groups (animal resting places, salt marshes, semideserts and steppes, shrublands, alpine screes, wet grasslands, water bodies and the subnival zone, Fig. 1).

Principal Component Analysis (PCA) was used to assess relationships between the growth form categories. Their relations to the eight habitat types was also assessed by their passive projection to the PCA ordination plane using pie graphs depicting the weighted proportion of the four types of space occupancy strategies (and annuals) in an average community. We further used a direct ordination analysis, Redundancy Analysis (RDA, ter Braak and Šmilauer, 1998), to relate growth form spectra to recorded environmental variables. The weighted proportion of individual growth forms in each relevé was used as response variables, and several recorded abiotic factors, altitude, substrate texture, soil stability, salinity, shading, soil moisture and fertility, as explanatory variables. Furthermore, an attempt was made to relate vegetation physiognomy (relative shrub, herb, and moss layer cover) and species composition characteristics to growth form spectra by their passive projection to the RDA ordination plane. Species composition was expressed by the first two DCA axes obtained from the indirect ordination of vegetation relevés. In RDA, we first reduced the number of predictors within the group of abiotic variables using a forward selection procedure, and then we performed several RDAs to assess their relative effects on growth form composition using a variance partitioning procedure, in which case the factors not used as predictors were defined as covariables to remove their effects and obtain the net effect of an individual factor (using this approach we constructed tests analogous to the testing of particular terms in ANOVA models but for multivariate data). Monte Carlo permutation tests (999 permutations) were used to assess the significance of the relationships found in the multivariate analyses. The ordination methods and permutation tests were conducted using CANOCO (ter Braak and Šmilauer, 1998). The distribution of abundant growth forms along the elevational gradient was visualized with response curves fitted using generalized additive models with a Poisson distribution and a stepwise selection of alternative models with increasing complexity up to 3 degree of freedom using the Akaike Information Criterion (see Lepš and Šmilauer 2003: 139).

Results

Implementation of the classification scheme

Based on a study of their morphology, 540 species of the East Ladakh flora were classified into 20 clonal growth forms (Fig. 2) and these categories were further grouped into four space occupancy strategies (Fig. 3). Some clonal growth forms, although morphologically distinct, were represented by only one or two species and several species were classified into more than one category (Figs. 4 and 5). With the exception of type 18 ("*Potamogeton amblyphyllus*", possessing turions) absent from one of the subregions (Table 1), and type 19 (represented only by *Potamogeton perfoliatus*, which propagates by stem fragments) absent from all subregions, all other clonal growth forms were found in all studied subregions. Potamogeton perfoliatus was found only in river beds of the Indus valley and was outside of the altitudinal ranges considered in this study, however we keep the category in our classification for possible study of an expanded geographical area. Classification of space occupancy strategies revealed that a quarter of the species are non-spreading integrators (25.8%). Non-spreading splitters are slightly less numerous (22.3%), followed by

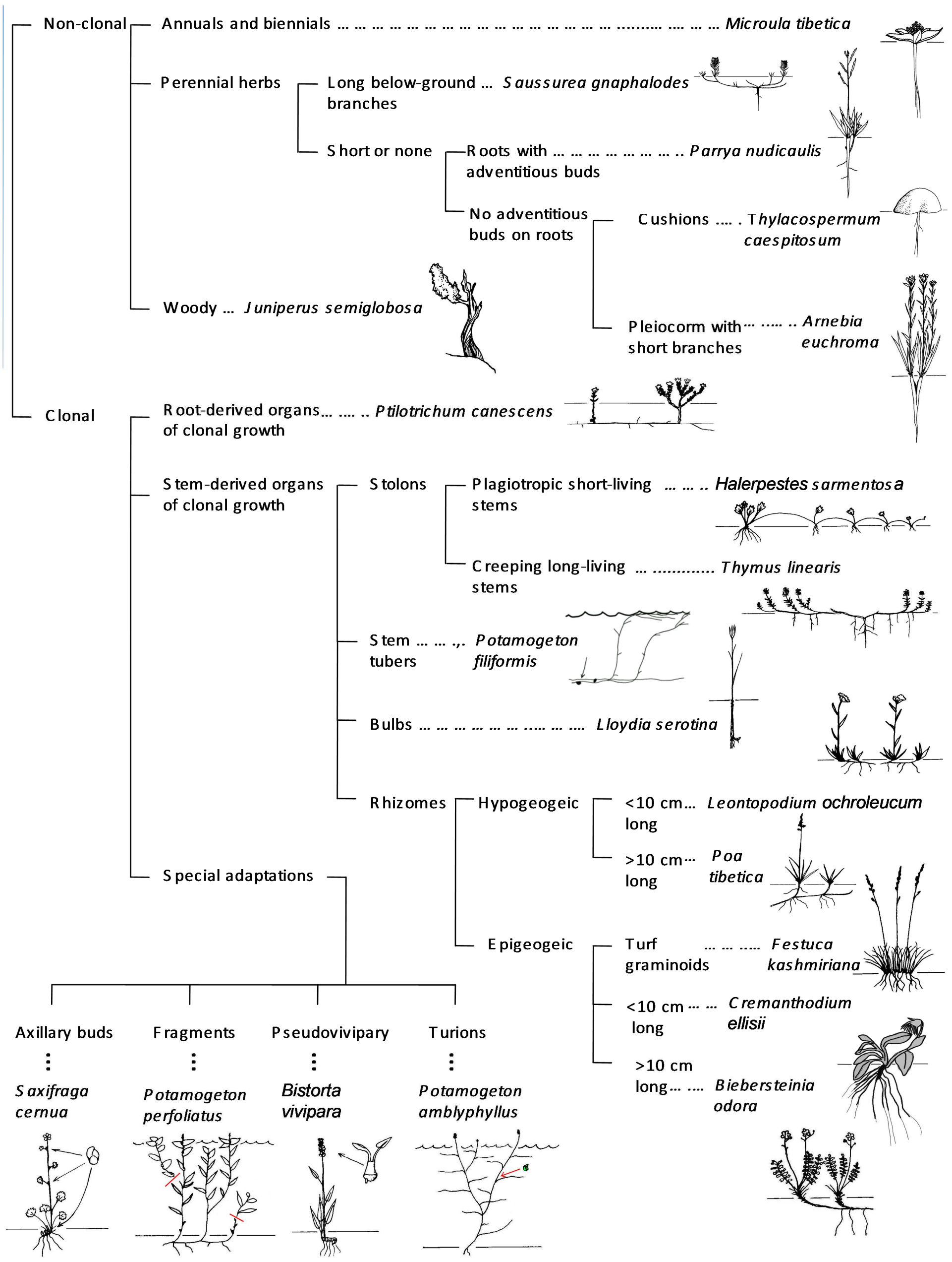

Fig. 2. Classification scheme of 20 growth forms of vascular plants in East Ladakh (NW Himalaya, India). Growth forms are named after representative species.

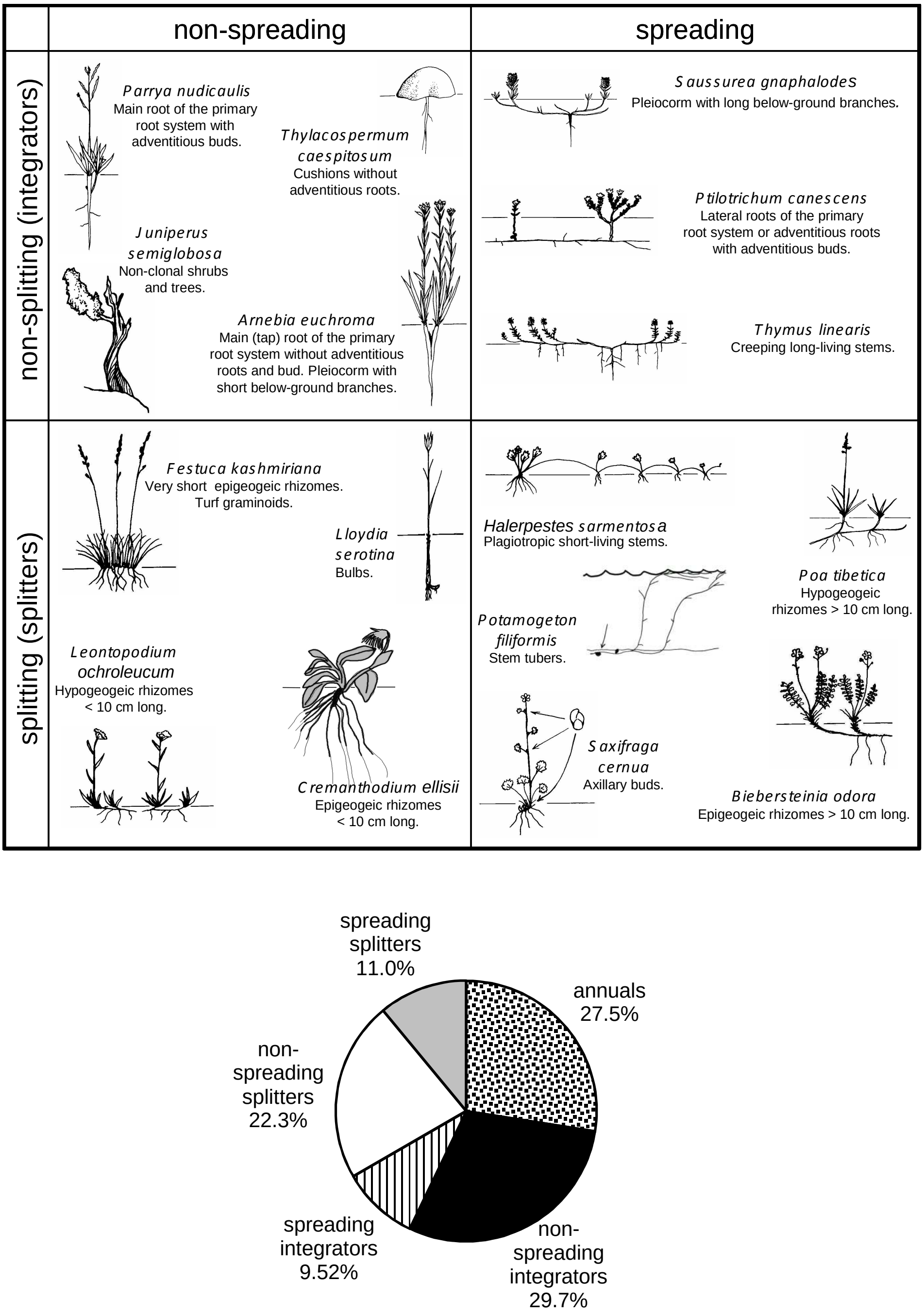

Fig. 3. Various forms of clonal structures categorized according to their capacity to spread and the longevity of their connections. The origin of the clonal structure and examples of species are given for each of the four categories.

spreading splitters (14.8%), and spreading integrators (9.5% of the flora) (Fig. 3).

Clonal growth spectra in the flora and plant communities

The analysis of the proportions of the different growth forms in the four study regions showed that non-clonal plants generally prevail (58.7% of the species, attaining a weighted average vegetation cover of 76% per plot) over clonal plants, and their proportion increased with altitude (Table 1). The most abundant non-clonal perennial plants were those with a tap root, a pleiocorm having short branches (“*Arnebia euchroma*” type), representing 22.9% of the vascular plants, prevailing in steppes (37.9% weighted cover), *Caragana* shrubs (28.1%) and screes (31.4%) (Table 2). Non-clonal perennial plants with a pleiocorm having long branches (“*Saussurea gnaphalodes*” type, 8.3% of the species) were significantly more abundant in alpine screes (25.9% cover). Annuals and biennials (“*Microula tibetica*” type, 24.5% species) prevailed in animal resting places (higher soil N and disturbances, 58% cover), and were also abundant in salt marshes (15.9%). Compact cushion plants (“*Thylacospermum caespitosum*” type, 3%) occurred exclusively in the subnival zone (12.5% cover). The most abundant clonal plants were those with very short epigeogenous rhizomes (“*Festuca kashmiriana*” type, 13.4% species) prevailing in moist *Kobresia* grasslands (32.8%), but also in steppes (25.6%) and shrubland (23.8%). Clonal plants with long (>10 cm) below-ground hypogeogenous rhizomes (“*Poa tibetica*” type, 7.4% of the species) prevailed in salt marshes (36%), representing extrazonal vegetation in valley basins, and were the least abundant in screes (5%) and steppes (8.6%). All other types occurred in less than 6% of the E Ladakh vascular plants.

Growth form categories showed similar habitat preferences when unweighted data and data weighted by abundance were considered (Table 2). There was a close similarity between the ranking of growth types across species and across data weighted by cover. Differences in the percentage of species belonging to a particular growth type between the weighted and unweighted data were found for plants with very short epigeogenous rhizomes (“*Festuca kashmiriana*” type) and plants with long hypogeogenous rhizomes (“*Poa tibetica*” type), i.e., clonal dominants in steppes and salt marshes, respectively, which are overrepresented in the vegetation data. However, plants with a pleiocorm having short branches (“*Arnebia euchroma*” type) and annual and biennial plants (“*Microula tibetica*” type) (both non-clonal plants) are relatively underrepresented in the vegetation data, indicating their inability to occupy space and dominate vegetation.

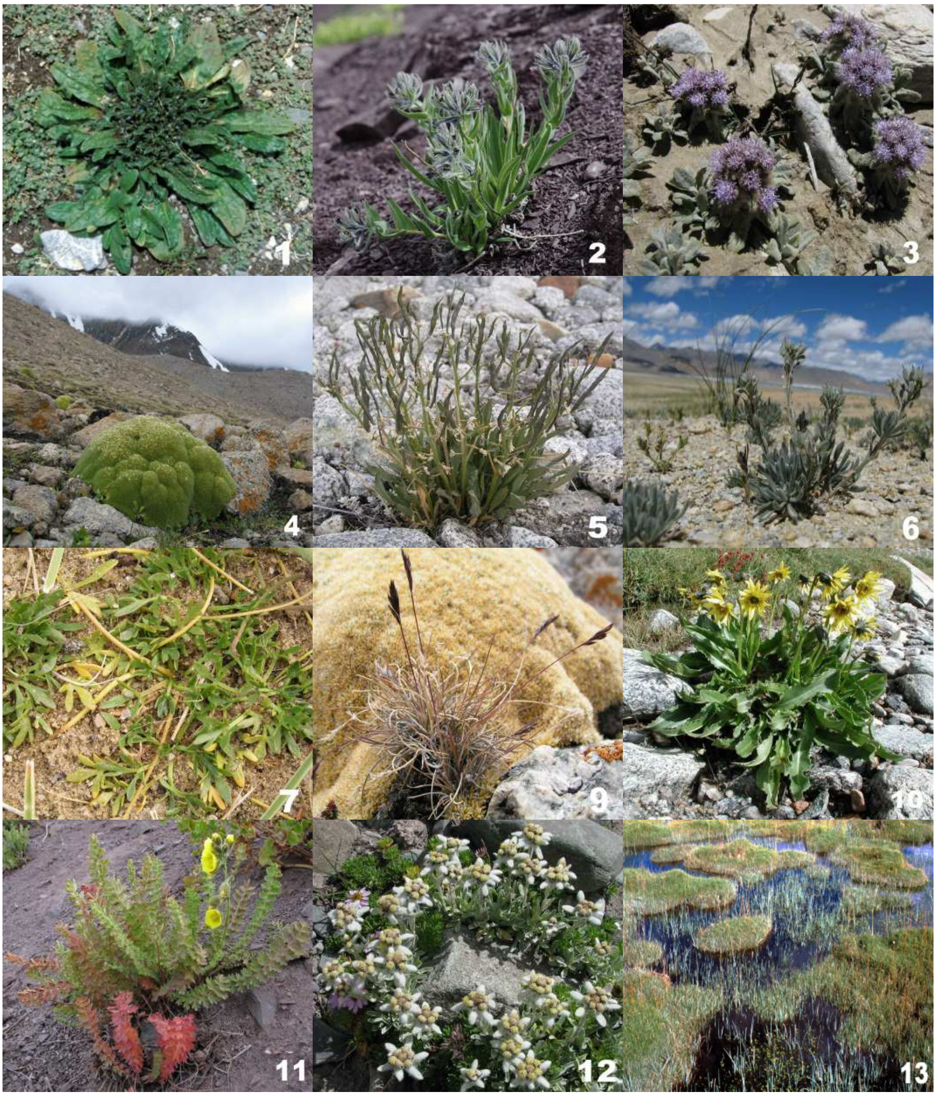

Fig. 4. Representative species of selected growth forms. 1 – *Microula tibetica*; 2 – *Arnebia euchroma*; 3 – *Saussurea gnaphalodes*; 4 – *Thylacospermum caespitosum*; 5 – *Parrya nudicaulis*; 6 – *Ptilotrichum canescens*; 7 – *Halerpestes sarmentosa*; 9 – *Festuca kashmiriana*; 10 – *Cremanthodium ellisii*; 11 – *Biebersteinia odora*; 12 – *Leontopodium ochroleucum*; 13 – *Poa tibetica*.

The co-occurrence of individual growth forms is shown in the PCA diagram (Fig. 6), together with passively projected centroids of the eight habitat types. Among growth forms that showed a positive correlation were plants with a

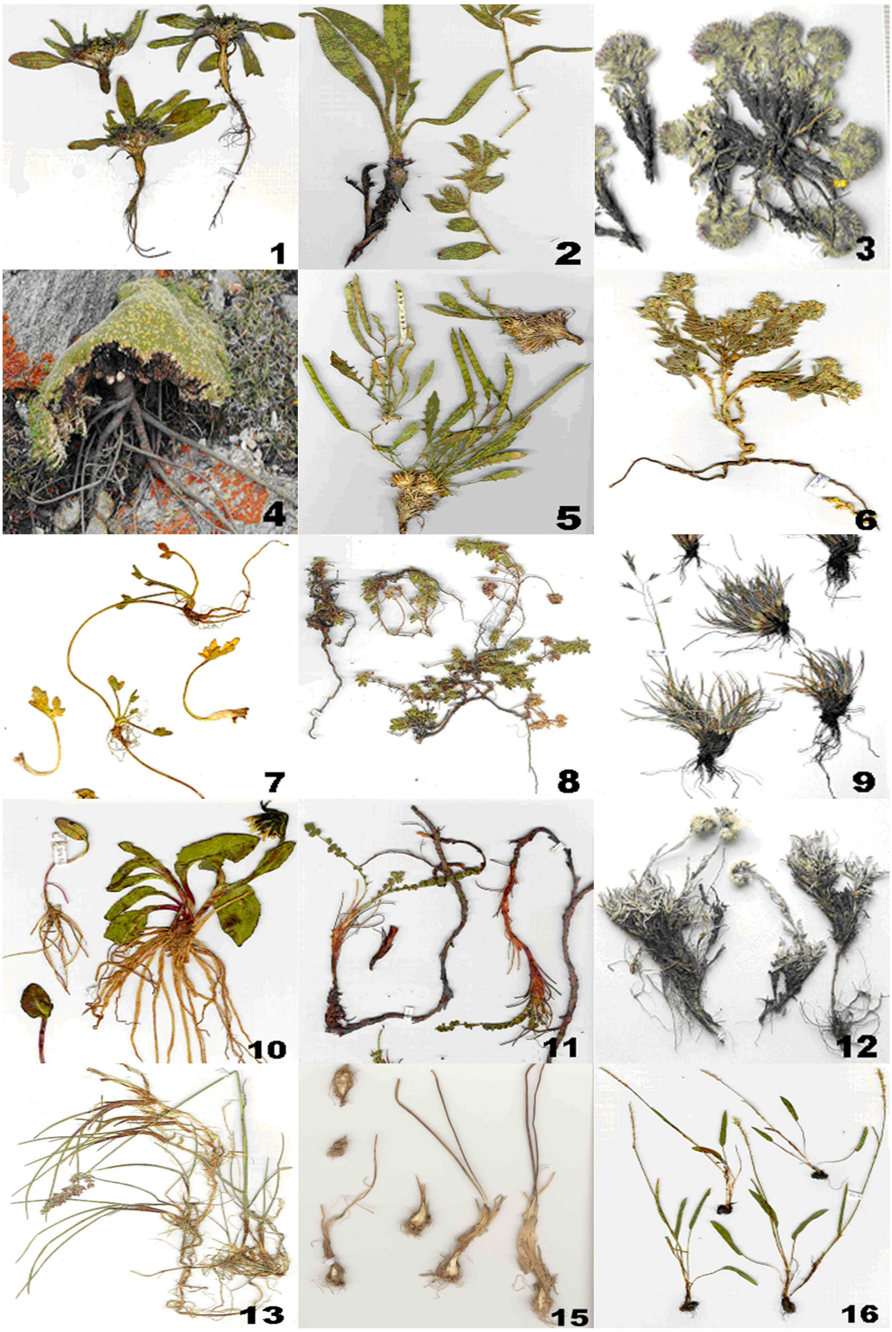

Fig. 5. Herbarium specimens of species representative of selected growth form categories. 1 – *Microula tibetica*; 2 – *Arnebia euchroma*; 3 – *Saussurea gnaphalodes*; 4 – *Thylacospermum caespitosum*; 5 – *Parrya nudicaulis*; 6 – *Ptilotrichum canescens*; 7 – *Halerpestes sarmentosa*; 8 – *Thymus linearis*; 9 – *Festuca kashmiriana*; 10 – *Cremanthodium ellisii*; 11 – *Biebersteinia odora*; 12 – *Leontopodium ochroleucum*; 13 – *Poa tibetica*; 15 – *Lloydia serotina*; 16 – *Bistorta vivipara*.

pleiocorm having short branches ("*Arnebia euchroma*" type), rootsprouters ("*Ptilotrichum canescens*" type) and woody plants ("*Juniperus semiglobosa*" type) and these were negatively correlated with plants possessing short rhizomes ("*Cremanthodium ellisii*" and "*Leontopodium ochroleucum*" types). A positive relationship was found between plants with a pleiocorm having long branches ("*Saussurea gnaphalodes*" type) and cushion plants ("*Thylacospermum caespitosum*" type).

The PCA diagram also shows the habitat-specific proportion of clonal structures categorized by their capacity to spread and the longevity of connections (clonal strategies of space occupancy). Of the 540 species recorded in the East Ladakh, the most abundant were non-spreading integrators (25.8%), prevailing in steppes (41.2% weighted cover), alpine screes (40.7%), *Caragana* shrubs (45.4%), and subnival vegetation (38.4%). The second most abundant were non-spreading splitters (22.3% of the total species number) prevailing in wet grassland (50.2% weighted cover). Spreading splitters (14.8%) dominated in water bodies (79.3%) and salt marshes (43.5%). Least represented were spreading integrators (9.5%) being relatively more abundant in salt marshes (15.9%).

Relationships among the growth form categories and environmental variables

Variation in growth form composition were further explored in the context of the site (abiotic variables used as explanatory variables) and vegetation characteristics (vegetation physiognomy and species composition passively projected into the RDA ordination plane) (Fig. 7). From the pool of 14 abiotic variables, 10 were selected by forward selection in the following order: moisture, altitude, salinity, stability, fertility, silt, shading, 1 cm gravel, rocks, slope, and sand. These explained 28.4% of the total variation in growth form composition (Table 2). The variance partitioning procedure revealed that moisture is the most important determinant of growth form spectra, explaining the most data variation (Table 3), followed by altitude, salinity, stability, fertility and shading, with all other factors explaining less than 1% of variability. The first RDA axis corresponded to the salinity and gravel gradients tightly correlating with the "*Poa tibetica*" (positively) and "*Arnebia euchroma*" (negatively) types, i.e., clonal plants with long hypogeogenous rhizomes prevail in more saline sites, whereas non-clonal plants with a tap root, a pleiocorm having short branches, increase in importance on steeper sites with larger gravel proportion. The silt and moisture gradients (corresponding to DCA axis 1 describing variation in species composition from dry semi-deserts and steppes to wet grasslands and water bodies), correlated positively with the proportion of stoloniferous plants ("*Halerpestes sarmentosa*" type) and plants with short epi- and hypogeogenous

Table 2. Proportions of 20 growth forms, unweighted (uw) and weighted-by-abundance data (w), in the entire dataset of 369 relevés and in eight major vegetation types. The numbers in brackets behind the proportions indicate the ranking of the growth forms in each vegetation type.

	All dataset			Salt marshes		Animal places		Water bodies		Shrubs		Steppes		Wet meadows		Screes		Subnival	
Mean / maximum elevation (m)				4592 / 5140		4801 / 5200		4695 / 5110		4636 / 5110		4732 / 5320		4775 / 5510		5057 / 5620		5243 / 5750	
Number of relevés/species		369/275		92/121		9/29		11/23		48/101		83/80		142/100		20/85		52/107	
Growth form	n	uw	w	uw	w	un	w	uw	w	uw	w	uw	w	uw	w	uw	W	Uw	w
1 – *Microula*	45	11.6	10.4	17.9 (3)	15.8 (3)	54.7 (1)	58 (1)	4.5 (5)	8 (4)	6.5 (5)	7.4 (5)	11.8 (3)	10 (4)	6.9 (3)	4.8 (6)	3.1 (7)	2.4 (7)	6.7 (6)	5.5 (6)
2 – *Arnebia*	76	24.5	21.3	11.8 (4)	8.4 (4)	6.5 (4)	3.5 (6)	0.9 (9)	0.4 (9)	35.1 (1)	28.1 (1)	40.5 (1)	37.9 (1)	16.3 (2)	12.3 (4)	32.5 (1)	31.2 (1)	25.4 (1)	24 (1)
3 – *Saussurea*	23	7.4	7.4	4.0 (7)	4.3 (7)	3.4 (5)	7.5 (3)	1.8 (8)	1.5 (7)	6 (6)	4.8 (7)	4.0 (6)	3.8 (6)	8.2 (5)	8.9 (5)	23.1 (2)	25.9 (2)	14.8 (3)	14 (4)
4 – *Thylacospermum*	10	2.9	2.6					0.9 (9)	0.4 (9)	0.9 (8)	0.6 (8)	0.5 (8)	0.5 (8)	1.5 (6)	1.2 (9)	8.1 (4)	8 (4)	14.1 (4)	12.5 (5)
5 – *Parrya*	2	0.2	0.3											0.1 (8)	0.1 (14)	0.4 (13)	0.2 (14)	1.5 (8)	1.9 (8)
6 – *Ptilorichum*	7	3.8	3.9	1.6 (8)	2 (9)	1.8 (6)	1.2 (7)			4.7 (7)	7 (6)	11.7 (4)	10.3 (3)	0.4 (8)	0.6 (11)	0.5 (12)	0.4 (13)	0.4 (9)	0.3 (10)
7 – *Halerpestes*	6	2.9	3.3	5.6 (5)	6.9 (5)			38 (1)	41.7 (1)					2.6 (7)	2.1 (8)			0.3 (10)	0.2 (11)
8 – *Thymus*	1	0.2	0.2							0.3 (9)	0.3 (10)			0.4 (8)	0.4 (13)	1.1 (9)	0.9 (12)		
9 – *Festuca*	37	21.0	22.0	21.4 (2)	18.8 (2)	8.1 (3)	3.5 (5)	2.7 (6)	2.9 (6)	24.9 (2)	23.8 (2)	20.4 (2)	25.6 (2)	28 (1)	32.8 (1)	17.3 (3)	16 (3)	18.3 (2)	18.5 (2)
10 – *Cremanthodium*	27	5.0	4.0	5.2 (6)	4.4 (6)			9.8 (4)	6.4 (5)	0.8 (7)	0.5 (9)	0.1 (9)	0.1 (10)	16.1 (2)	12.8 (3)	4.7 (5)	3.8 (6)	5.0 (7)	3.8 (7)
11 – *Biebersteinia*	2	0.1	0.2							0.3 (9)	0.2 (11)					1.1 (11)	1.8 (9)	0.3 (10)	0.2
12 – *Leontopodium*	4	1.5	1.5	1.9 (8)	2.2 (8)			2.3 (7)	1.1 (8)	0.3 (9)	0.2 (11)			4.7 (6)	4.6 (7)	1.2 (10)	1 (11)	0.9 (8)	0.9 (9)
13 – *Poa*	22	15.3	18.4	29.2 (1)	36 (1)	21.9 (2)	18.9 (2)	19.7 (2)	11.4 (3)	9.9 (4)	10.4 (4)	8.5 (5)	8.6 (5)	12.6 (3)	17.2 (2)	3.5 (6)	5 (5)	12 (5)	17.9 (3)
14 – *Potamogeton*	1	0.2	0.3	0.1 (11)	0.03 (12)	3.4 (5)	7.4 (4)					0.5 (8)	0.3 (9)						
15 – *Lloydia*	1	0.1	0.1													1.9 (8)	1.9 (8)	0.1 (12)	0.08
16 – *Bistorta*	1	0.1	0.1											0.6 (8)	0.6 (10)				
17 – *Saxifraga*	1	0.04	0.02											0.03 (8)	0.02 (15)			0.2 (11)	0.15
18 – *Potamogeton*	1	0.7	1.0	0.4 (10)	0.6 (10)			19.2 (3)	26.3 (2)					0.26 (8)	0.42 (12)				
20 – *Juniperus*	8	2.2	3.2	0.7 (9)	0.53 (11)					10.1 (3)	16.7 (3)	1.8 (7)	2.7 (7)	1.26 (6)	1.24 (9)	1.3 (9)	1.3 (10)		

rhizomes ("*Cremanthodium ellisii*" and "*Leontopodium ochroleucum*" types), and negatively with the proportion of root sprouters ("*Ptilotrichum canescens*" type) and non-clonal perennial plants with a pleiocorm having short below-ground branches ("*Arnebia euchroma*" type). The altitudinal gradient (corresponding to DCA axis 2 describing compositional changes from salt marshes in valley basins towards alpine screes and subnival vegetation at highest elevations) correlated positively with cushion plants ("*Thylacospermum caespitosum*" type), and plants with a pleiocorm having long branches ("*Saussurea gnaphalodes*" type), and negatively with annuals and biennials ("*Microula tibetica*" type) preferring sandy sites at lower elevations. The RDA diagram also shows the clonal strategies of space occupancy passively projected into the ordination plane (Fig. 7), with non-spreading integrators prevailing in shaded rocky slopes, spreading integrators at the highest elevation, non-spreading splitters in stable grassland along water streams, and spreading splitters in saline and nutrient-rich environments at lower elevations.

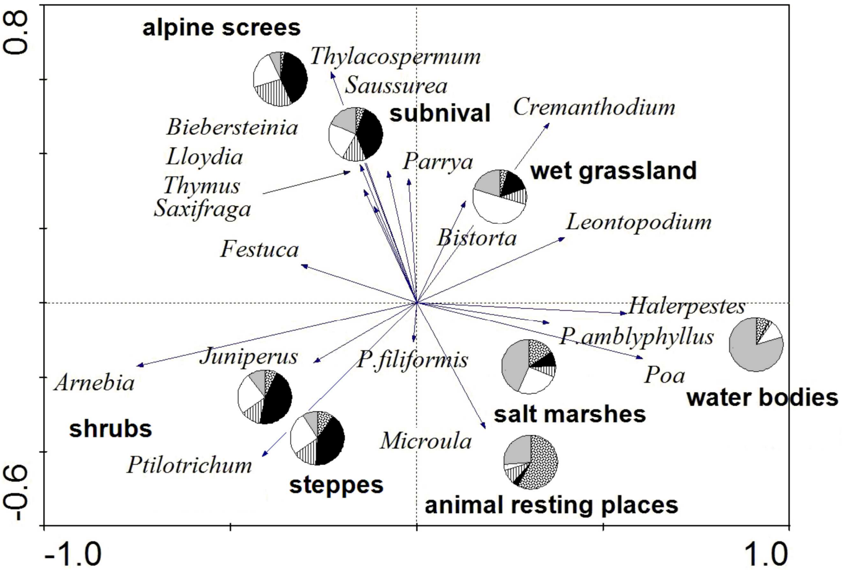

Fig. 6. Relations between the growth form categories, named after representative species, in a PCA plot with passively projected supplementary variables: eight major vegetation types in E Ladakh and additional information on weighted proportions of annuals and four clonal structures categorized according to their capacity to spread and the longevity of their connections (black – non-spreading integrators, white – non-spreading splitters, grey – spreading splitters, hatched – spreading integrators, dotted – annuals).

Modelling of both unweighted data and data weighted by abundance with altitude showed similar patterns, therefore only results from the latter approach are presented (Fig. 8). Non-clonal annual and biennial plants (“*Microula tibetica*” type) were more frequent at lower elevations than non-clonal perennial plants having a tap root and below-ground branches of varying length (“*Arnebia euchroma*” and “*Saussurea gnaphalodes*” types) (Fig. 8). At the cold end of the gradient, i.e., at higher elevations, non-clonal cushion plants (“*Thylacospermum* *caespitosum*” type) dominated. Most clonal growth forms occurred at middle elevations from 4500 to 5500 m a.s.l. (Fig. 8). Clonal plants with long

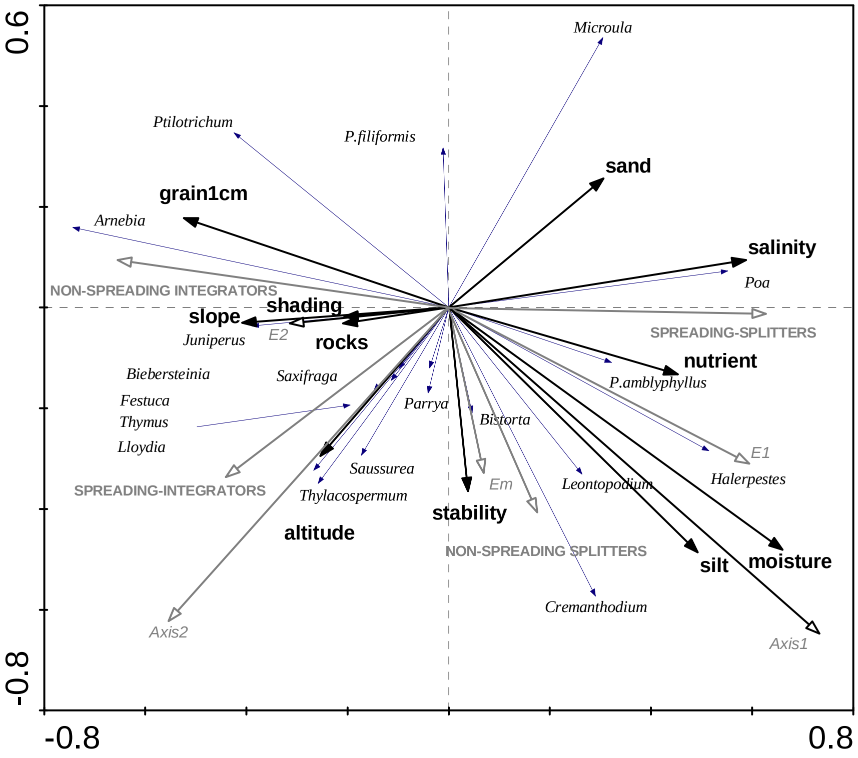

Fig. 7. Relations between recorded environmental variables and growth form categories in an RDA plot with passively projected supplementary variables: vegetation physiognomy (relative shrub, herb, and moss layer cover), species composition (first two DCA axes), and four basic clonal growth strategies (centroids).

hypogeogenous rhizomes ("*Poa tibetica*" type) or above-ground stolons ("*Halerpestes sarmentosa*" type), root sprouters ("*Ptilotrichum canescens*" type), and woody plants ("*Juniperus semiglobosa*" type) decline with altitude, while non-clonal perennial plants with a pleicorm and long below-ground branches ("*Saussurea gnaphalodes*" type), cushion plants ("*Thylacospermum caespitosum*" type), and clonal plants with short epigeogenous rhizomes ("*Cremanthodium ellisii*" type) increase with altitude. Annual and biennial plants ("*Microula tibetica*" type), non-clonal perennial plants with a pleiocorm having short branches ("*Arnebia euchroma*" type), and plants with very short epigeogenous rhizomes ("*Festuca kashmiriana*" type) peaked at middle elevations, and decreased towards both lower and higher elevations.

Table 3. RDA analyses of growth form compositional variability. The explained variability and significance levels (*** – P<0.001, ** – P<0.01, * – P<0.05) are shown for the marginal effects – variability explained by a given factor without considering other factors, and the partial (net) effects – variability explained by a given factor after accounting for the effects of other factors (co-variables).

	Marginal		Partial	
Variables	*F*	%	*F*	%
Abiotic	12.89***	28.4		
Altitude	20.10***	5.2	14.87***	4
Stability	8.59***	2.4	7.42***	2.1
Moisture	48.78***	11.7	14.11***	4.8
Salinity	29.82***	7.5	9.24***	2.6
Fertility	18.25***	4.7	5.31***	1.5
Shading	4.98***	1.3	4.74***	1.3
Slope	15.45***	4	2.17*	0.6
Rocks	4.05**	1.1	2.34*	0.7
>20 cm	14.94***	3.9	1.25	0.4
5 cm	27.89***	7.1	1.53	0.4
1 cm	24.33***	6.2	3.52**	1
Sand	12.28***	3.2	2.21*	0.6
Silt	29.68***	7.5	1.25	0.4
Loam	25.38***	6.5	1.12	0.3

Discussion

We distinguished 20 clonal growth forms and four space occupancy strategies responding to multiple environmental gradients, mainly moisture and altitude together with several soil variables, in West Himalaya. Overall, in dry and the most elevated communities non-spreading integrators prevailed (i.e., plants with a pleiocorm having short branches, and cushion plants), in wet grasslands nonspreading splitters (plants with short rhizomes) and in saline wetlands spreading splitters prevailed (plants with long hypogeogenous rhizomes).

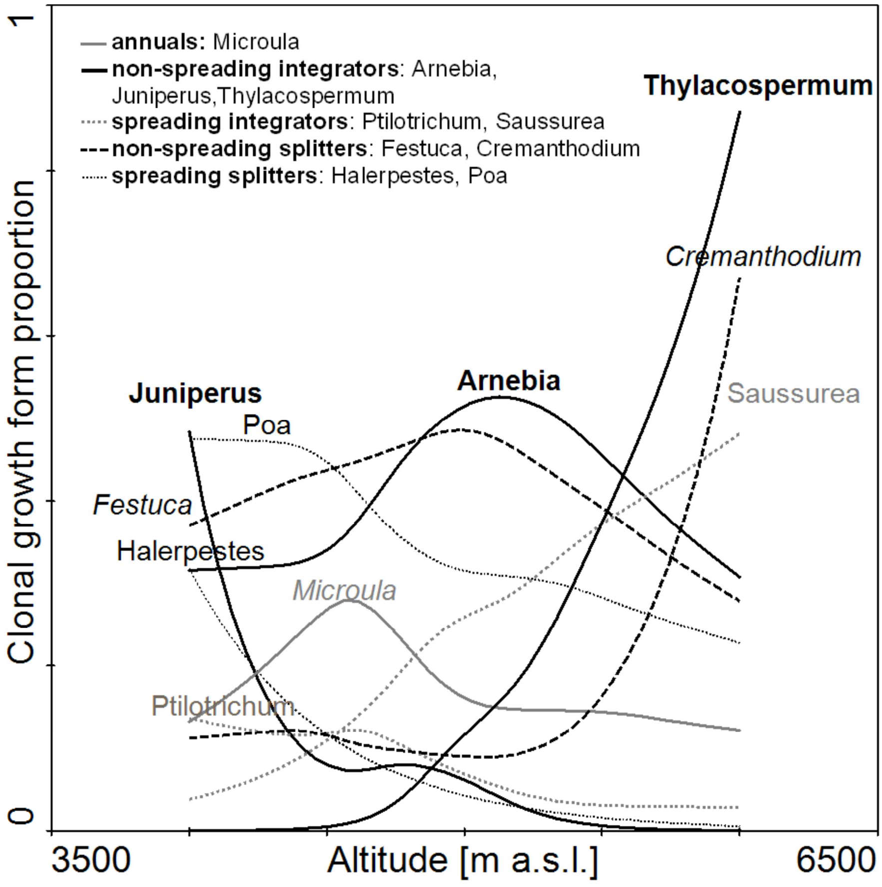

Fig. 8. Response curves of selected growth forms for the altitudinal gradient named after typical representatives. Growth form proportions were weighted by species cover within each vegetation type.

Spreading integrators (plants with a pleiocorm having long branches, and root sprouters) were the least frequent strategies concentrated in places with an unstable substrate (screes and alluvial deposits). These overall results point out that future studies aiming to compare the relevance of clonality in different regions should take multiple environmental gradients into account.

Classification

Due to the high diversity of existing plant forms, inevitably some doubts arise during the process of attributing species to a given growth form. In our case, it was not clear whether below-ground stem branches emerging from the main root in some species (e.g., *Ephedra gerardiana*, *Waldheimia tridactylites*, and *Thylacospermum caespitosum*) could, when disconnected from the main root by disturbance, form adventitious roots and survive independently and therefore show some restricted capacity of clonal growth. *Physochlaina praealta*, a species forming tubers, seems to be potentially able to form rhizomes in sandy soil. Cushion growth forms, although they may be formed by species that produce adventitious roots and thus might be considered clonal in other regions of the world (Rauh, 1939), are mostly represented by non-clonal plants in the region. Data from other regions are necessary to test whether the cushion growth form is more important for plant life in extreme environments than below-ground morphology. Phenotypic plasticity and variability at the population level and in relation to environmental conditions may require further study focusing on individual species and their ecology.

As the studied region was the first to which our methodology was applied outside of Central Europe, we can only make a general comparison with the flora of Central Europe (Klimešová and Klimeš, 2008) and some preliminary comparisons with other regions. A remarkable difference when comparing the presented results with Central Europe, is the high proportion of non-clonal plants with a perennial main root in Ladakh, which is in contradiction with general statements about the predominance of clonal plants in cold alpine and arctic regions (Klimeš et al., 1997; Callaghan et al., 1997). Whereas alpine communities of the German Alps and the arctic tundra of central Spitsbergen host about 30% of non-clonal species (Klimešová, unpublished), the studied region of Ladakh includes about 70% of non-clonal taxa. Also in the "*Carex-Elyna*" plant community studied by Hartmann in the Alps (1957) clonal plants prevail, reaching 86.8% out of 228 species in the community. Although the regions differ in precipitation and solar irradiance, another possible reason for these differences can be found in the species pools of these regions, which show a higher proportion of dicot species in Ladakh.

In a previous study (see Klimeš, 2003) the low proportion of taxa with long rhizomes was interpreted as caused by substrate instability due to cryoturbation. Cryopedogenic processes and shifting of screes would probably affect clonal plants with long rhizomes ("*Poa tibetica*" type) more than non-clonal plants with a pleiocorm having long branches ("*Saussurea gnaphalodes*" type). A similar non-clonal growth form with a perennial main root and long below-ground stems called "Schopftriebe" was described from unstable screes in the

Alps by Hess (1909) and resemble those growing in Ladakh (see "*Saussurea gnaphalodes*" type). Its success is because scree has surface layers that are not very suitable for rooting due to mechanical stress and lack of fine soil particles and thus shoots broken off by shifting of scree are not able to form new roots and subsequently die. A main root, however, with attached perennial stem bases bearing a bud bank growing in deep and more stable strata of scree is able to rebuild new shoots. When the mechanical stress caused by frost and thaw cycles is too high, hardly any adaptation is possible (Jonasson and Callaghan, 1992).

In comparison to other regions of the world where clonality is well known, e.g., Central Europe, where the two most common clonal growth forms with hypogeogenous and epigeogenous rhizomes are equally represented (Klimešová and Klimeš, 2008), hypogeogenous rhizomes are much less common in Ladakh. Because hypogeogenous rhizomes are the most common at wet sites elsewhere (van Groenendael et al., 1996), the prevailing dryness of the Trans-Himalayan environment may be a reason for this difference, and restriction of this type to extra-zonal wetlands confirms this idea.

In contrast to central Europe, Ladakh also has a very low number of species forming adventitious buds on horizontal roots ("*Ptilotrichum canescens*" type). However, the habitats that they occupy are similar to the habitats where rootsprouters grow in the Alps: glaciofluvial deposits (Stöcklin and Bäumler, 1996). Deep homogenous substrates with occasional disturbance by water erosion seem to be very suitable for this clonal growth form.

The high number of annual species in the studied region resembles dry lowland areas (Baitulin, 1979), however in Ladakh they are represented by weedy plants at low elevations affected by nutrient enrichment from domestic animals and not ephemeral species that profit from short periods with sufficient water supply. Contrary to lowlands, dry regions have a low proportion of bulbous and tuberous geophytes, which is, however, reported from other mountainous regions as well (Körner, 1999a). The factors responsible for this might be frost heaving and cryoturbation typical of nival zones with repeating thaw and freezing cycles.

When grouping the clonal growth forms into four categories of space occupancy strategies we can support earlier analyses (van Groenendael et al., 1996; Jónsdóttir and Watson, 1997; Halassy et al., 2005; Klimeš, 2008; Sosnová et al., 2010), in which correlations between spreading splitters and moisture and non-spreading integrators and dryness were indicated. In our case moisture was correlated with nutrient and salinity as well, and dryness was correlated with poorly developed soils on slopes and rock. This tendency, observed in a whole range of habitats from wetlands (Sosnová et al., 2010) to semi-deserts (this study) is evolutionarily constrained, as monocots lacking a main root have inherited a splitting strategy and at the same time they generally prefer wetter habitats (van Groenendael et al., 1996). The importance of both elevation and moisture gradients in our analysis indicates that when comparing clonal growth spectra between regions the position on the moisture gradient of the regions should be taken into account.

According to Grace (1993), clonal growth organs differ in function and are therefore filtered by environmental factors into different communities. Our results support this idea, because certain types of clonal growth forms were preferred in certain habitats. Preliminarily, we can propose key functions of some clonal growth types, e.g., amelioration of the environment in cushion plants, searching for water in plants with a perennial main root; anchorage in unstable substrates in plants with a pleiocorm having long branches; fast colonization of sandy and gravel deposits along rivers by horizontal roots with adventitious buds, and space occupancy by long hypogeogenous rhizomes in wetlands. The proposed preferences remain to be tested in other alpine regions of the world. We believe that the clonal growth form identification key and the hierarchical nature of the introduced scheme will certainly allow non-experts to apply these plant functions and make meaningful comparisons between different mountain regions.

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Appendix 1

Identification key

1 Stems only above-ground..2

1 Stems also below-ground..7

2 Neither adventitious roots nor buds present on roots (non-clonal plants)...................................3

2 Horizontal rooting stem...4

2 Plant fragments...5

3 **Annual and biennial herbs** (“*Microula tibetica*” type)

3 **Trees** (“*Juniperus semiglobosa*” type)

3 Cushion plants

Cushion plants:

(“*Thylacospermum caespitosum*” type)

4 Horizontal stems short-lived (up to two growing seasons)

Short-lived horizontal rooting stems on or above soil surface:

Short-lived herbaceous clonal growth organ rooting in the soil and providing connection between offspring plants or formed by creeping axis; nodes on stem bearing leaves, internodes usually long, stem serving as a storage organ and bud bank; vegetative spreading usually fast and persistence low (“*Halerpestes sarmentosa*” type)

4 Horizontal stems long-lived (more than two growing seasons)

Long-lived horizontal rooting stems on soil surface:

Long-lived, usually woody clonal growth organ formed by creeping axis, rooting in the soil; nodes of youngest parts bearing leaves, internodes shorter than in short-lived horizontal stems; stem serving as a storage organ and bud bank; vegetative spreading slow and persistence high (“*Thymus linearis*” type)

5 Plant fragments specialized for overwintering (turions)

Turions:

Detachable overwintering buds (usually in water plants) composed by tightly arranged leaves filled with food reserves; turions, developing axially or apically and needing vernalization for re-growth (“*Potamogeton amblyphyllus*” type)

5 Unspecialized plant fragments

Plant fragments of stem origin:

Detached parts of shoot with rooting ability ("*Potamogeton perfoliatus*" type)

5 Detachable offspring..6

6 Offspring in inflorescence (pseudovivipary)

Plantlets (pseudovivipary):

Meristem normally develops into a flower, but forms vegetative buds (plantlets, bulbils, root or stem tubercules), which are sometimes soon detached from the parent plant; alternatively the entire inflorescence falls off and the plantlets root at the soil surface; offspring size similar to seedlings ("*Bistorta vivipara*" type)

6 Offspring in axils of leaves

Axillary buds:

Small vegetative diaspores produced in axils of leaves on above-ground stems formed by axillary buds subtended by small storage organ of stem, root (tubercule) of leaf (bulbil) origin; buds soon shed from mother plant, beginning to grow immediately; resembling seedlings in size ("*Saxifraga cernua*" type)

7 Below-ground stems lacking adventitious roots and roots lacking adventitious buds.......................8

7 Below-ground stems possessing adventitious roots and/or roots possessing adventitious buds......9

8 Long below-ground stems (pleiocorms)

Pleiocorm having long branches:

Plant possessing a primary root system lacking adventitious roots and buds; tap root serving as storage organ; bud bank situated on perennial stems with long (more than 10 cm) branches serving as vascular link between shoots and primary root; non-clonal plants ("*Saussurea gnaphalodes*" type)

8 Short below-ground stems (pleiocorms)

Pleiocorm having short branches:

Plant possessing a primary root system lacking adventitious roots and buds; tap root serving as storage organ; bud bank situated on perennial stems with short (less than 10 cm) branches serving as vascular link between shoots and primary root; non-clonal plants ("*Arnebia euchroma*" type)

9 Roots with adventitious buds..10

9 Roots without adventitious buds...11

10 Adventitious buds on main root

Main root with adventitious buds:

Main root (including hypocotyle) forming adventitious buds spontaneously or after injury; clonal growth usually only after fragmentation of main root ("*Parrya nudicaulis*" type)

10 Adventitious buds on horizontal creeping roots

Horizontal roots with adventitious buds:

Branches of main root and adventitious roots forming adventitious buds spontaneously or after injury; roots serving as bud bank and vascular connection between offspring shoots; lateral spread usually extensive; persistence differing among species ("*Ptilotrichum canescens*" type)

11 Below-ground stems lacking specialized storage organs..12

11 Below-ground stems possessing specialized storage organs, stems sometimes reduced...........15

12 Stems formed below-ground (hypogeogenous rhizomes)..13

12 Stems formed at soil surface and older parts placed below-ground (epigeogenous rhizomes).....14

13 Hypogeogenous rhizomes with short increments (less than 10 cm)

Short hypogeogenous rhizomes:

Perennial organs of stem origin formed below-ground; rhizome usually growing at a species-specific depth, periodically becoming orthotropic and forming above-ground shoots; horizontal part of rhizome bearing bracts, some roots possessing short internodes; vegetative spreading intermediate; persistence differing considerably among species ("*Leontopodium ochroleucum*" type)

13 Hypogeogenous rhizomes with long increments (more than 10 cm)

Long hypogeogenous rhizomes:

Perennial organs of stem origin formed below-ground; rhizome usually growing at a species-specific depth, periodically becoming orthotropic and forming above-ground shoots; horizontal part of the rhizome bearing bracts, some roots possessing long internodes; vegetative spreading fast; persistence differing considerably among species ("*Poa tibetica*" type)

14 Short epigeogenous rhizome

Short epigeogenous rhizomes:

Perennial organ of stem origin formed above-ground; its distal part covered by soil and litter or pulled into the soil by contraction of roots; nodes bearing green leaves; internodes usually short; rhizomes bearing roots and serving as bud bank and storage organ; vegetative spread low (up to one cm per year); persistence usually low; typical of tussock grasses ("*Festuca kashmiriana*" type)

14 Medium epigeogenous rhizome

Medium long epigeogenous rhizomes:

Perennial organ of stem origin formed above-ground; its distal part covered by soil and litter or pulled into the soil by contraction of roots; nodes bearing green leaves; internodes usually short; rhizomes bearing roots and serving as bud bank and storage organ; vegetative spread low (up to a few cm per year); persistence usually low ("*Cremanthodium ellisii*" type)

14 Long epigeogenous rhizomes

Long epigeogenous rhizomes:

Perennial organ of stem origin formed above-ground; its distal part covered by soil and litter or pulled into the soil by contraction of roots; nodes bearing green leaves; internodes usually short; rhizomes bearing roots and serving as bud bank and storage organ; vegetative spread low (up to a few cm per year); persistence usually long, resulting in a large preserved rhizome system ("*Biebersteinia odora*" type)

15 Storage in leaves

Bulbs:

Storage and perennation organ consisting of storage leaves and shortened stem base; stem providing bud bank and connection between offspring shoots; lateral spread low ("*Lloydia serotina*" type)

15 Storage in stem

Stem tubers:

Below-ground, usually short-lived storage and regenerative organ of shoot origin; lateral spread high in the case of offspring tubers formed on hypogeogenous rhizomes, persistence usually low ("*Potamogeton filiformis*" type)

CHAPTER IV

Cushions of *Thylacospermum caespitosum* (Caryophyllaceae) do not facilitate other plants under extreme altitude and dry conditions in the north-west Himalaya

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Photo on previous page: *Thylacospermum caespitosum* (Caryophyllaceae) in Rupshu region, E Ladakh.

Cushions of *Thylacospermum caespitosum* (Caryophyllaceae) do not facilitate other plants under extreme altitude and dry conditions in the north-west Himalaya

Abstract

Cushion plants are commonly considered as keystone nurse species that ameliorate the harsh conditions they inhabit in alpine ecosystems, thus facilitating other species and increasing alpine plant biodiversity. A literature search resulted in 25 key studies showing overwhelming facilitative effects of different cushion plants and hypothesizing greater facilitation with increased environmental severity (i.e. higher altitude and/or lower rainfall). At the same time, emerging ecological theory alongside the cushion-specific literature suggests that facilitation might not always occur under extreme environmental conditions, and especially under high altitude and dryness. To assess these hypotheses, possible nursing effects of *Thylacospermum caespitosum* (Caryophyllaceae) were examined at extremely high altitude (5900 m a.s.l.) and in dry conditions (precipitation 100 mm year^{-1}) in Eastern Ladakh, Trans-Himalaya. This is, by far, the highest site, and the second driest, at which the effects of cushions have been studied so far. In accordance with the theoretical predictions, no nursing effects of *T. caespitosum* on other alpine plants were detected. The number and abundance of species were greater outside cushions than within and on the edge of cushions. None of the 13 species detected was positively associated with cushions, while nine of them were negatively associated. Plant diversity increased with the size of the area sampled outside cushions, but no species–area relationship was found within cushions. The results support the emerging theoretical prediction of restricted facilitative effects under extreme combinations of cold and dryness, integrating these ideas in the context of the ecology of cushionplants. This evidence suggests that cases of missing strong facilitation are likely to be found in other extreme alpine conditions.

Key words: Alpine plants, *Thylacospermum caespitosum*, Caryophyllaceae, biodiversity, competition and facilitation, stress, nurse plant, Tibet, Ladakh, Himalaya.

Introduction

It is often assumed that the distribution and abundance of species in high-elevation communities is enhanced by the presence of neighbours, where interactions are predominantly facilitative (Callaway et al., 2002). In these high-altitude regions, positive interactions involve nurse plants facilitating the establishment of other species within their canopy by offering microhabitats that are more favourable for seed germination and/or seedling recruitment than the surrounding environment (Cavieres and Badano, 2009).

Cushion plants are expected to be among the most effective nurse species in alpine habitats (Arredondo-Núñez et al., 2009) and have long fascinated ecologists (Rauh, 1939; Körner, 2003). Their low and compact growth form acts as an efficient heat and water trap and represents a clear model of evolutionary convergence across phylogenetically unrelated taxa in different regions of the world (Körner, 2003; Sklenář, 2009). It has been repeatedly reported that cushion plants act as nurse species in different alpine conditions, where significantly more plant species grow within cushions than outside their canopy. For example, a literature search of studies assessing potential nursing effects of cushions on other plants resulted in 25 key publications (see Table 1 in Chapter V of this thesis, page 98), all showing predominantly facilitative effects. Much of this evidence comes from high-Andean communities (but see Antonsson et al., 2009; Haussmann et al., 2010; Yang et al., 2010). The positive effects of cushions were greater with increased environmental severity (Arredondo-Núñez et al., 2009; Badano et al., 2010), i.e. at higher altitudes (Cavieres et al., 2002; Fajardo et al., 2008; Antonsson et al., 2009; Yang et al., 2010) and under drier conditions (Cavieres et al., 2006).

The greater nursing effect under more severe habitats supports the hypothesis of increased facilitation under more stressful conditions (Callaway et al., 2002). At the same time, ecological theory on species interactions alongside the cushion-specific literature also suggests that the importance of facilitative effects could ultimately decrease under extreme stressful conditions (Michalet et al., 2006). Recent studies suggest that the outcome of interspecific interactions should be positive only at intermediate levels of environmental severity or, at least, not increase constantly with growing abiotic stress (Kaway and Tokeshi, 2007). Under extreme abiotic conditions plants could derive little benefit, and still incur a cost, from growing in close proximity to neighbours (Maestre et al., 2005). Such theoretical predictions suggest that the combination of multiple stress factors, particularly cold and dry conditions, could override any advantage originating from facilitation (Maestre et al., 2009). These hypotheses remain, however, mostly theoretical (Kaway and Tokeshi, 2007), and sometimes controversial (Gross et al., 2010), probably due to the lack of field evidence from really extreme environmental conditions. Specifically, the real outcome on species interactions under the combination of extreme cold and dry conditions largely remains to be demonstrated. There is some evidence that facilitation in extremely dry environments may be limited (Maestre et al., 2005). Similarly, in the context of the cushion-specific literature, Cavieres and Badano (2009) showed the first evidence indicating less positive effects of cushions under drier environmental conditions. Le Roux and McGeoch (2010) tested for a possible decrease in the facilitation of cushions with increasing altitude and wind exposure. They never found negative nursing effects in the conditions with the greatest stress, possibly because the region receives high precipitation (see Table 1 in Chapter V of this thesis, page 98). We hypothesize that the absence of negative effects with increasing habitat severity in the cushion-specific literature may have been caused by the lack of studies under a combination of both extremely cold and dry conditions.

The region of Ladakh, in the westernmost part of the Tibetan Plateau (Trans-Himalaya), offers particularly suitable environmental conditions for testing these hypotheses. Ladakh is often referred to as a 'cold desert', presenting a combination of extremely cold conditions and marked water scarcity. Compared with other Trans-Himalayan regions, Ladakh lies in the rainshadow of the Himalaya. Owing to its position, which poses a barrier to seasonal monsoons, the whole area receives very low precipitation (<100 mm year^{-1}; Dvorský et al., 2011). The dominant cushion species in the study area is *Thylacospermum caespitosum* (Caryophyllaceae). It occupies the highest vegetation belts at the upper vegetation limits in the region (Klimeš and Doležal, 2010; Klimešová et al., 2011) and it is basically the only species with a sufficiently large canopy to host other plant individuals. This species is phylogenetically related to another cushion species, *Arenaria polytrichoides*, with a demonstrated nursing effect in the Himalaya, due to improved soil resources (Yang et al., 2010). Here, the hypothesis that *T. caespitosum* is a nurse plant for other alpine species was studied. The possible effects of this cushion species depending on its size were also assessed assuming that effects of cushions could be detected by different species-area relationships within and outside cushions (Cavieres and Badano, 2009). A location was chosen which is at the highest distribution of *T. caespitosum* in the region (at 5900 m a.s.l.), close to the upper altitudinal limits of vascular plants, in what is probably the most stressful combination of environmental conditions under which cushion species effects have been assessed so far. Our results show that the role of cushion species as nurse species might not be as general as expected, especially under very extreme environmental conditions.

Methods

The survey was carried out in the Lunglung valley, on the western slope of the Chalung Mountains east of Lake Tso Moriri (33°05′N, 78°27′E), in the same study site as described in Klimeš and Doležal (2010). The highest peaks in the area are 6400 m a.s.l. and the upper vegetation limit in the valley is at 6030 m. However, most species, including *Thylacospermum caespitosum*, do not often occur above 5900 m. *Thylacospermum caespitosum* accounts for most of the vegetation cover in this vegetation type (Dvorský et al., 2011), which totals around 10 %. The mean air temperature at the soil surface was around -8 °C (with the minimal and maximal temperatures ranging between –22 °C and +9 °C; Klimeš and Doležal, 2010).

Sampling was carried out at a homogenous site with a gentle slope where 50 well-spaced, not rotten, *T. caespitosum* cushions of varying size (diameter of 2 cm up to 72.5 cm) were randomly selected. No water sources or brooks were detectable at the site. For each cushion, species composition (occurrence and number of individuals) within, at the edge of and outside each cushion was surveyed. A similar area was sampled for the three positions considered for each cushion (i.e. within, at the edge of and outside cushions). For the 'outside' position, a 'belt' was defined surrounding the sampled cushion plant, with the belt having the same area as the cushion (using the formula of circular rings, i.e. the outer radius of the belt was equal to $r \times \sqrt{2}$, with r being the cushion radius). For the edges, a similar approach was followed where for each belt an approximately similar area was sampled within and outside the cushion. A total of 150 sampling units were surveyed (50 cushions × 3 positions). For tussock species, such as *Poa attenuata*, each tussock was considered to be a separate individual.

For the data analyses two parallel approaches were followed. First, to test the effect of cushions on other plants, resampling techniques were used, as commonly applied in studies considering nursing cushion effects (Cavieres et al., 2002, 2006; Yang et al., 2010). We used the Friedman test, with post-hoc comparisons, based on 9999 Monte-Carlo resamplings (Hollander and Wolfe, 1999). This is a non-parametric testa ssessing symmetry of responses across treatments for repeated measurements. The three positions (i.e. within, at the edge of and outside cushions) were considered as repeated measures for each individual cushion. The test was applied to assess if the total number of species and the total number of individuals were associated with any specific position. The same test was applied to individual species (in terms of both species occurrence and number of individuals). Then, to assess the possible effects of this cushion species depending on its size, we fitted linear models explaining the number of species and the number of individuals with the size of cushions, the sampling position and their interaction. The three positions were again considered as repeated measures for each individual cushion. Parametric and non-parametric (i.e. permanova; Anderson, 2001) models gave largely similar results. The interaction of size × position in these models indicated different slopes of fitted regression lines using a standardized major axis (Warton et al., 2006). The linear models described above were also applied to test the effect of cushion size on individual species. All analyses were run in R (R Development Core Team, 2009).

Results

A total of 13 species (Table 1) were found in the 150 sampling units considered. In terms of diversity, the number of species and individuals increased (as expected) with sample area (Fig. 1).

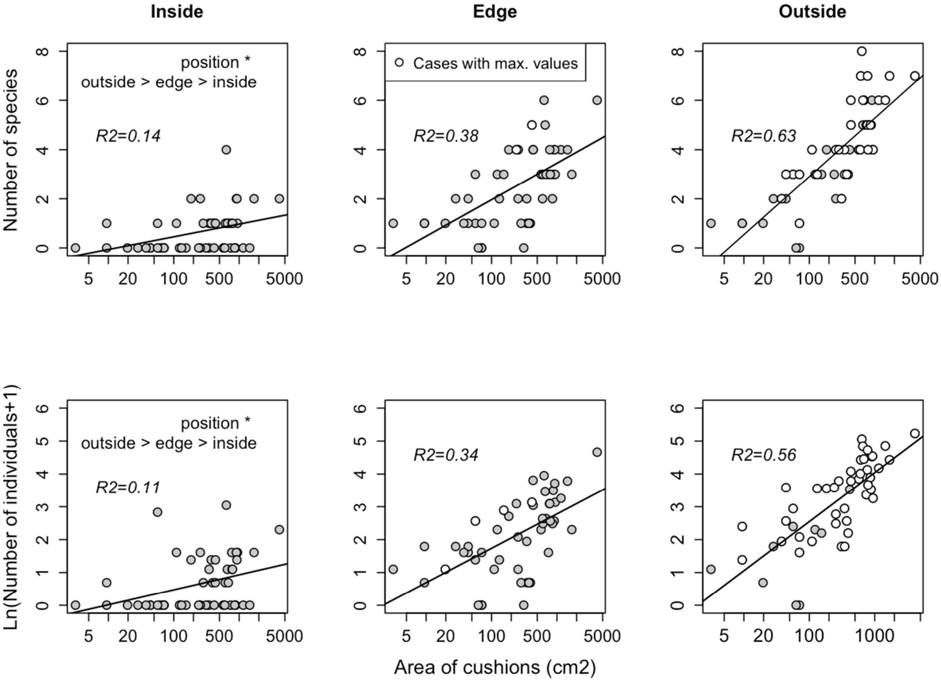

Fig. 1. Variation in the number of species and individuals (after log-transformation) with sample size within, at the edge of and outside cushions of *T. caespitosum*. Open circles refer to the position, for each cushion, where the maximum number of species or individuals was reached (see text for detail). For both number of species and number of individuals there was a significant effect of position ($P<0.05$), with post-hoc tests between positions indicating outside > edge > inside. The R^2 values of fitted lines are given for each panel.

The increment was, however, stronger outside cushions (higher variance explained outside cushions and a significant shift in regression slopes according to standardized major axis regressions). The numbers of species and individuals were never higher within a given cushion; they were higher either outside (more frequently) or on the edge of cushions (Fig. 1).

None of these species was positively associated with the cushion species, in terms of either occurrence or number of individuals found. On the contrary, nine out of 13 species were negatively associated with *T. caespitosum*, being more frequently found outside the cushions. For each individual cushion, species generally reached lower abundance inside it (Table 1 and Supplementary Data Figure S1). Only four species, i.e. three *Saussurea* species and *Stellaria depressa*, showed, in some cases, the highest abundance within individual cushions. These cases were rather occasional for *Saussurea* sp. (possibly a previously undescribed species) and *Stellaria depressa*. The other two species did not show clear associations with any position. Only *Saussurea gnaphalodes* was limited to the cushions, but with one individual in 150 sampling units.

Discussion

Our study provides a concrete example of no nursing effect of a high-elevation cushion plant (*T. caespitosum*) growing under extreme environmental conditions. Compared with the existing literature on cushion species (see Table 1 in Chapter V of this thesis, page 98), and on facilitation in general, we selected a site presenting the most stressful conditions, particularly possessing the most extreme combination of cold and dry conditions. In such extreme conditions, we did not find any evidence of positive effects of *T. caespitosum* on other plants, supporting the theoretical predictions advocating for a decrease of facilitation under extreme stressful conditions (Michalet et al., 2006; Maestre et al., 2009). Negative, or non-positive, effects of cushion species have rarely been reported and, as far as we know, never so clearly. Cavieres and Badano (2009), for example, did not find any location with negative effects of cushions on local diversity, while negative effects of cushions are generally observed on a minority of species at a given site (Arredondo-Núñez et al., 2009; Yang et al., 2010). Fajardo et al. (2008) found an equal number of positive and negative associations at their lowest site,
but almost exclusively positive associations at their highest site. Similarly, le Roux and McGeoch (2010) found some negative associations, but only at their lowest site. As such our results provide new evidence that needs to be taken into account when generalizing existing theories, and testing new hypotheses, on nursing effects of cushions under extreme alpine conditions. As the entire

Species	N° sampling units present			N° sampling units x cushions with highest abundance			Mean relative proportion of individuals			Occurrence				N° individuals			Preferred position
	inside	edge	outside	inside	edge	outside	inside	edge	outside	inside vs. edge	outside vs. edge	outside vs. inside	Area	inside vs. edge	outside vs. edge	outside vs. inside	
Aphragmus oxycarpus	1	4	14	0	3	14	0.4%	2.8%	3.9%		*	*			*	*	outside > edge > inside
Draba altaica	4	30	40	0	7	36	12.6%	29.9%	41.5%	*	*	*	*	*	*	*	outside > edge > inside
Draba oreades	0	9	12	0	2	11	0.0%	9.1%	7.0%	*		*		*	*	*	outside & edge > inside
Eritrichium hemisphaericum	0	2	12	0	1	12	0.0%	0.4%	1.5%	*	*	*	*	*	*	*	outside > edge > inside
Poa attennuata	7	30	38	0	12	30	15.9%	26.4%	19.0%	*	*	*	*	*	*	*	outside > edge > inside
Saussurea glacialis	6	5	8	4	1	6	20.0%	4.6%	3.5%								ns
Saussurea gnaphaloides	1	0	0	1	0	0	4.3%	0.0%	0.0%								ns
Saussurea sp.	8	26	33	3	12	24	28.1%	14.9%	13.3%	*	*	*	*	*	*	*	outside > edge > inside
Saxifraga cernua	0	0	1	0	0	1	0.0%	0.0%	0.0%								ns
Saxifraga nanella	0	2	2	0	2	2	0.0%	0.4%	0.4%				*				ns
Saxifraga stella-aurea	0	0	4	0	0	4	0.0%	0.0%	0.3%		*	*	*	*	*	*	outside > edge & inside
Stellaria decumbens	0	7	14	0	3	14	0.0%	3.1%	3.1%	*	*	*	*	*	*	*	outside > edge > inside
Stellaria depressa	5	10	10	1	1	10	18.4%	7.9%	6.5%	*		*	*	*		*	outside & edge > inside

Table 1. Species found and their distribution inside, at the edge of and outside cushions of *T. caespitosum*. Resampling with post-hoc comparisons was used to define the preferred position of a species (*indicates P < 0.05; ns P > 0.05). 'Area' effects indicate increase of species occurrence with sample size.

gradient of stressful conditions on high mountains throughout the world has probably not been studied so far, cases of missing facilitation in extremely stressful conditions are likely to be found elsewhere. Although the present study is based on a single cushion species, it is unlikely that the results are due to specific features of this particular cushion species, especially because *T. caespitosum* is phylogenetically and morphologically related to *A. polytrichoides* which has demonstrated a positive nursing effect in the Himalaya (Yang et al., 2010). In comparison with Yang et al. (2010), our site does not receive summer monsoons, is characterized by much lower precipitation (approx. 700 mm year^{-1} vs. approx. 100 mm year^{-1} in our site) and is situated at a much higher altitude (>1200 m higher).

Why were facilitative effects by cushions not developed under these extreme conditions? It is possible that in these extreme environments all species in the species pool are stress tolerant, i.e. they might be sufficiently adapted to the harsh conditions they inhabit, even without nursing effects (Michalet et al., 2006). In extremely arid habitats in particular, stress-tolerant species do not necessarily profit from facilitative effects from other species but compete for limited resources (Maestre et al., 2009). Ameliorative effects of cushion plants are said to include improved temperature conditions, an increased growing season and greater availability of resources (Yang et al., 2010). However, soil crusts, instead of cushion species, can provide microhabitats that are favourable for seed germination and/or seedling recruitment (Gold and Bliss, 1995), with the additional advantage of not having to compete with cushions. Soil crusts are frequent in alpine and sub-nival zones of different Ladakh regions and often occupy areas devoid of vascular plants (Řeháková et al., 2011). Moreover, in contrast to other regions of the world, the growing season in the study area is extremely short (<2 months; Klimeš and Doležal, 2010) and cushions probably cannot help much in prolonging it. Finally, although the effect of cushions on species with different traits has been considered (Arredondo-Núñez et al., 2009; Yang et al., 2010), little is known about the effects of cushions with different traits on species interactions. Thus, although *T. caespitosum* is largely similar to many other studied cushion species, the extreme compactness of its branches could partially inhibit settling of other plants. Considering the life histories of the interacting species (Maestre et al., 2009), together with the effect of different cushion growth forms (e.g. Rauh, 1939), could provide new insights to generalize cushion effects on other alpine species. However, this may be limited, at present, by two constraints. First, as far as we know, besides the preliminary work by Rauh (1939), a common set of traits for cushion species has not been agreed. Secondly, given the preponderance of positive associations found in the literature, meta-analyses of existing data probably cannot reveal any effect of the growth form of cushions on other species.

In conclusion, our results provide evidence that cushionplants do not act as nurse species in extreme alpine conditions, suggesting that cases of missing strong facilitation are likely to be found elsewhere. More generally, our study brings new evidence of the emerging theoretical predictions that the outcome of plant interactions can strongly depend on the combination of temperature and water stress. As such, this study supports the theoretical prediction that facilitation should not hold under extreme combinations of these two factors (Maestre et al., 2009), integrating these emerging ideas in the context of the ecology of cushion plants.

Supporting information

Additional supporting information may be found in the online version of this article:

Figure S1 Variation in the number of individuals (after log-transformation) with sample size within, at the edge of and outside cushions of *T. caespitosum*.

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CHAPTER V

Testing the stress-gradient hypothesis at the roof of the world: effects of the cushion plant *Thylacospermum caespitosum* on species assemblages

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Photo on previous page: a view of western Tibet from Shukule II Peak (6400 m a.s.l.), Rupshu region, E Ladakh.

Testing the stress-gradient hypothesis at the roof of the world: effects of the cushion plant *Thylacospermum caespitosum* on species assemblages.

Abstract

Many cushion plants ameliorate the harsh environment they inhabit in alpine ecosystems and act as nurse plants, with significantly more species growing within their canopy than outside. These facilitative interactions seem to increase with the abiotic stress, thus supporting the stress-gradient hypothesis. We tested this prediction by exploring the association pattern of vascular plants with the dominant cushion plant *Thylacospermum caespitosum* (Caryophyllaceae) in the arid Trans-Himalaya, where vascular plants occur at one of the highest worldwide elevational limits. We compared plant composition between 1112 pair-plots placed both inside cushions and in surrounding open areas, in communities from cold steppes to subnival zones along two elevational gradients (East Karakorum: 4850–5250 m and Little Tibet: 5350–5850 m). We used PERMANOVA to assess differences in species composition, Friedman-based permutation tests to determine individual species habitat preferences, species-area curves to assess whether interactions are size-dependent and competitive intensity and importance indices to evaluate plant-plant interactions. No indications for net facilitation were found along the elevation gradients. The open areas were not only richer in species, but not a single species preferred to grow exclusively inside cushions, while 39–60% of 56 species detected had a significant preference for the habitat outside cushions. Across the entire elevation range of *T. caespitosum*, the number and abundance of species were greater outside cushions, suggesting that competitive rather than facilitative interactions prevail. This was supported by lower soil nutrient contents inside cushions, indicating a resource preemption, and little thermal amelioration at the extreme end of the elevational gradient. We attribute the negative associations to competition for limited resources, a strong environmental filter in arid high-mountain environment selecting the stress-tolerant species that do not rely on help from other plants during their life cycle and to the fact the cushions do not provide a better microhabitat to grow in.

Introduction

The response of alpine and subnival plants to recent climate warming and their potential distributional shifts along elevational gradients may, to a large extent, hinge on interactions between species (Brooker, 2006). With rising temperature, many species from the lower alpine zone have been recently shown to extend their range limits toward the subnival zone (Pauli et al., 2007; Erschbamer et al., 2011; Gottfried et al., 2012). This process can be facilitated or hindered by resident species. Newly arrived species to upper alpine zones do not need to be well adapted to harsh conditions and their survival may depend on the presence of safe microsites (Cavieres et al., 2005). Among protected (nurse) habitats which could potentially provide better growing conditions within the alpine zone, are canopies of dominant cushion plants. These low-growing and compact species represent one of the most suitable survival strategies in the alpine and arctic zones. Here, plants must cope with multiple stress factors including low temperature, strong wind and abrasion, unstable substrate and solifluction, and low nutrient and water availability (Körner, 2003). Under such conditions, the main mechanisms behind the facilitative processes could be thermal amelioration (Arroyo et al., 2003; Cavieres et al., 2006), water and nutrient provision (Núñez et al., 1999; Cavieres et al., 2006; Yang et al., 2010) and protection from strong desiccant winds by cushion plants (Hager and Fagi, 1990; Núñez et al., 1999; Cavieres et al., 2006; Yang et al., 2010).

Many cushion plant species have been shown to ameliorate the harsh environment and enable other plants to establish, survive and perform better in environments where they would otherwise not succeed (Nuñez et al., 1999; Badano et al., 2002; Cavieres et al., 2002; Acuña-Rodríguez et al., 2006; Cavieres et al., 2006; Antonsson et al., 2009; Cavieres and Badano, 2009; Haussmann et al., 2010). For example, a literature search of studies assessing potential nursing effects of cushions on other plants resulted in 27 key publications (Table 1), all showing predominantly facilitative effects. The research on positive plant interactions with cushion species significantly helped to verify the socalled stress-gradient-hypothesis (SGH). This model predicts that the outcome of plant interactions depends on the severity of the physical environment, with positive effects (facilitation) being more important in stressful environments and resource competition prevailing in less harsh conditions (Bertness and Callaway, 1994).

The positive role of cushion plants in enhancing the local community diversity has been mainly documented in temperate alpine and arctic conditions. Conversely, recent studies conducted in arid and semi-arid mountains (Maestre et al. 2005, 2006; Cavieres et al., 2006; Lortie and Callaway, 2006; de Bello et al., 2011) reported non-positive or negative interactions under extreme conditions of low temperature and water deficit (Cavieres et al., 2006; Fajardo et al., 2008; Cavieres and Badano, 2009). This supported the theoretical prediction that high stress could minimise the importance of facilitative effects by exclusively selecting the stress-tolerant species that do not rely on help from other plants during their life cycle. A debate between ecologists (Maestre et al., 2005, 2006; Lortie and Callaway, 2006) resulted in a refined SGH (Maestre et al. 2009) in which the predicted results of plant-plant interactions are specified with respect to the type of abiotic stress (resource vs. non-resource limitation) and the relative strategy of the participating species (competitive vs. stress tolerant) (Michalet, 2007). The positive role of cushion plants has been mostly documented in mountains with elevations below 5000 m and with an abundant water supply (Núñez et al., 1999; Badano et al., 2006; Antonsson et al., 2009; Cavieres and Badano, 2009; Sklenář, 2009). The type of plant interactions that can be expected under higher elevations (5000–6000 m) and aridity (<100 mm year^{-1}) is, however, largely unknown. Such conditions exist in the dry mountains of Trans-Himalaya, where vascular plants grow continuously up to 6000 m. Recently, we have documented that cushions of *Thylacospermum caespitosum* (Caryophyllaceae) do not facilitate other plants at extremely high elevations (5900 m) in the dry mountains of eastern Ladakh (de Bello et al., 2011). These findings led us to hypothesise that positive interactions between species could be more prominent under moderately stressful than extreme conditions (Holmgren and Scheffer, 2010). This called for further testing of the cushion's nursing effect along its entire distribution range.

Here we tested the nursing effect of cushion species *Thylacospermum caespitosum* (Cambess.) Schischkin (Caryophyllaceae) by assessing plant associations along an unprecedented elevational gradient (4850–5850 m) in the arid mountains of NW Himalayas. Despite the importance of cushion plants in alpine ecosystem (Hager and Fagi, 1990; Núñez et al., 1999; Badano et al., 2002; Cavieres et al., 2002; Acuña-Rodríguez et al., 2006; Cavieres et al., 2006; Antonsson et al., 2009; Cavieres and Badano, 2009; Haussmann et al., 2010 Yang et al., 2010), their impacts and functions require further attention, especially in remote mountain regions such as the Himalayas. This high altitude region is being strongly impacted by climate change and is experiencing rapid changes in biodiversity (Xu et al., 2009). These changes in biodiversity can alter ecosystem processes and the resilience and resistance of ecosystems to environmental change (Körner, 2003). Without baseline data on plant-plant interactions, however, we cannot track the effects of climate change, and without an understanding of the drivers of community assembly, we cannot predict how climate change may affect these highaltitude plant communities.

Table 1. List of studies assessing facilitative effects from cushion species on other plants. For each study, the table indicates the cushion species that was studied, the altitudinal range considered (in m a.s.l.), the study region, the approximate mean yearly rainfall (mm year^{-1}) and the effect detected (‘ + ’ indicates facilitation, ‘-’ indicates negative effects).

Study	**Year**	**Species**	**Altitude**	**Region**	**Rainfall**	**Effect**
Pyšek & Liška	1991	*Sibbaldia tetrandra*	3800	Pamiro-Alai, 42°N	2500	+
Nuňez et al.	1999	*Mulinum leptacanthum, Oreopolus glacialis*	1550-1600	Andes, 41°S	1000	+
Badano et al.	2002	*Oreopolus glacialis*	1900	Andes, 37°S	2300	+ but species specific
Cavieres et al.	2002	*Bolax gummifera*	700-900	Andes, 50°S	900-1000	+ more evident in higher altitudes
Arroyo et al.	2003	*Azorella monantha*	700-900	Andes, 50°S	900-1000	+
Cavieres et al.	2005	*Azorella monantha*	3100-3300	Andes, 33°S	943	+
Acuña-Rodriguez et al.	2006	*Laretia acaulis*	2800	Andes, 33°S	400-900	+
Badano et al.	2006	*Azorella monantha*	3580-3630	Andes, 33°S	>900	+
Badano & Cavieres	2006a	*Adesmia subterranea, Azorella monantha, A. madreporica*	3200-4000	Andes, 30-33°S	242-900	+
Badano & Cavieres	2006b	*Adesmia subterranea, Azorella madreporica, A. monantha, Discaria nana, Laretia acaulis, Mulinum leptacanthum, Oreopolus glacialis, Pycnophyllum bryoides*	1600-4400	Andes, 23-41°S	no data	+
Cavieres et al.	2006	*Laretia acaulis*	2800-3200	Andes, 33°S	445-943	increased facilitation at lower (drier) site
Zoller & Lenzin	2006	*Eritrichium nanum*	2170-3320	Alps	2170-3320	+
Cavieres et al.	2007	*Laretia acaulis, Azorella monantha*	2800-3600	Andes, 33°S	400-900	+
Badano & Marquet	2008	*Azorella monantha*	3580-3630	Andes, 33°S	>900	+
Fajardo et al.	2008	*Azorella madreporica*	3580-3630	Andes, 33°S	943	both + and -
Antonsson et al.	2009	*Silene acaulis*	1150-1447	Scandes, 68°N	839	+ above a given altitudinal threshold
Arredondo-Núňez et al.	2009	a meta-analysis	700-4400			+
Badano & Marquet	2009	*Azorella madreporica*	3400	Andes, 33°S	>900	+
Cavieres & Badano	2009	*Adesmia subterranea, Azorella madreporica, A. monantha, Discaria nana, Laretia acaulis, Mulinum leptacanthum, Oreopolus glacialis, Pycnophyllum bryoides*	900-4400	Andes, 23-50°S	42-1117	always + but lower effects at both extremes of the environmental severity gradient
Haussmann et al.	2009	*Azorella selago*	300	Marion Island, 46°S	2000	+
Quiroz et al.	2009	*Azorella madreporica*	3200-3580	Andes, 33°S	400	+ changes with altitude
Sklenář	2009	*Azorella dicranoides, A. corymbosa, Eudema nubigena, Hypochaeris* sp., *Xenophyllum humile*	4650	Andes, 0°S	no data	+
Badano et al.	2010	*Azorella madreporica, Laretia acaulis*	2700-3600	Andes, 33°S	400-900	+ increases with stress (- only at lower elevation)
Haussmann et al.	2010	*Azorella selago*	300	Marion Island, 46°S	2000	+
Yang et al.	2010	*Arenaria polytrichoides*	4500-4700	Himalaya, 28°N	680-790	+ increases with altitude
de Bello et al. (Chapter IV)	2011	*Thylacospermum caespitosum*	5900	Transhimalaya, 33°N	100	-
Anthelme et al.	2012	*Azorella aretioides*	4400, 4550, 4700	Andes, 0°S	1000	- lower elevation, + upper elevation
Dvorský et al. (this study)	2013	*Thylacospermum caespitosum*	4850-5850	Transhimalaya, 33°N	100	-

We investigated which of the two contrasting predictions would apply: would the nurse effect take place, as in most of the key studies on cushions worldwide, or would we find no facilitation as implied by the refined stress-gradient hypothesis? Specifically, we asked the following questions: 1) Are other vascular plants positively or negatively associated with cushions of *T. caespitosum*? 2) Does the effect of *T. caespitosum* on the surrounding vegetation depend on its size? 3) Does *T. caespitosum* influence the surrounding abiotic environment (temperature and soil physico-chemical properties)? 4) Do these effects and patterns change with elevation and water availability?

Methods

Study region

The study region is situated in Ladakh, Jammu & Kashmir State, India (Fig. 1). The area is a part of the Trans-Himalaya, being delimited by the Eastern Karakoram Range in the north and by the Great Himalaya Range in the south. Due to its position in the rain-shadow of the Himalaya Range, the region is arid and receives very little precipitation (<100 mm year^{-1}) (Hartmann, 1983; Wang, 1988). Evaporation exceeds precipitation at lower and middle elevations. At elevations above 5300 m, precipitation tends to increase (Miehe et al., 2001), however above 5600 m, water may remain unavailable due to soil water freezing.

To test the generality of plant interactions, we analysed the associations between *T. caespitosum* and other vascular plants from the cold steppes up to the subnival zones in two mountain ranges (Eastern Karakoram, Little Tibet). These differ in overall glaciation and hence have different upper distributional limits of vascular plant existence (Klimeš and Doležal, 2010; Dvorský et al., 2011). The two locations enabled us to test the SGH along an elevational gradient, mainly associated with decreasing temperature (Fig. 2), which together covered the entire elevation range of *T. caespitosum* cushions in Ladakh. This is correlated with a significant decrease in the mean annual/summer temperatures (from -1.6/7.7 °C to -10.4/4.4 °C between 4850–5850 m).

The first locality was a side valley near the village of Tiggur in Nubra Valley, which is situated in the northern part of Ladakh (34°45´N, 77°35´E) and belongs geomorphologically to the Eastern Karakoram Range (with the highest peak of Saser Kangri 7672 m, 15 km north of our study site). The relief is characterised by sharp and rugged ridges. Valleys are narrow and steep and regularly end in glaciers, the fronts of which usually start at c. 5300 m.

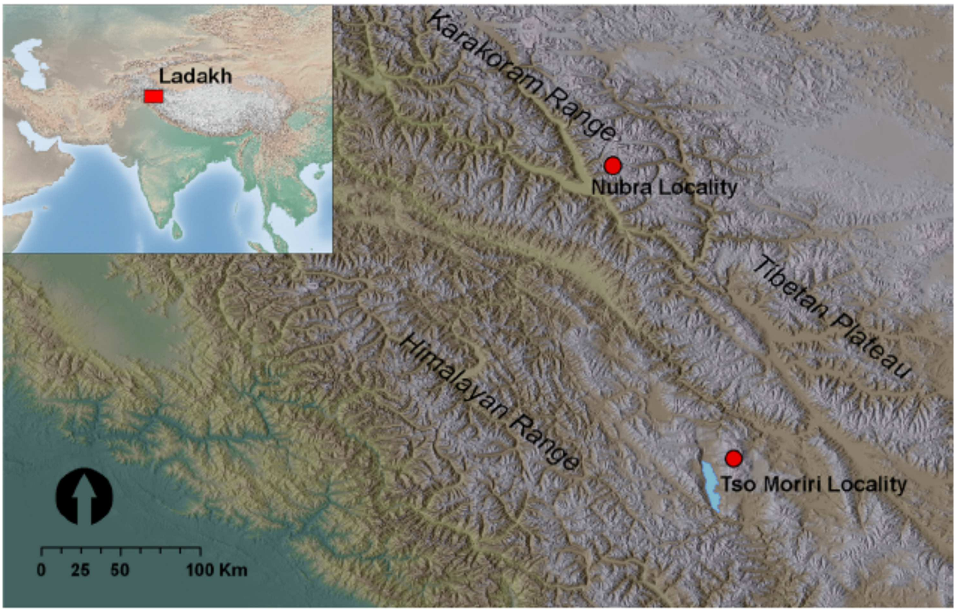


Fig. 1. Location of the two study localities. Nubra is situated within E Karakoram Range, Tso Moriri belongs to Tibetan Plateau.

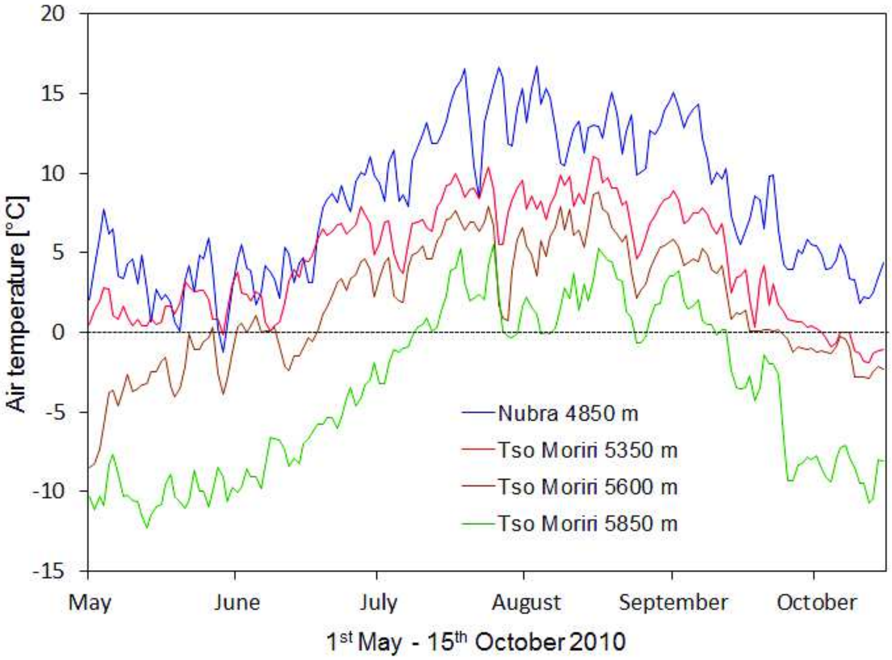


Fig. 2. Changes in air temperature regime with increasing elevation. Shown are mean daily air temperatures during the summer season of 2010 for selected Nubra and Tso Moriri sites.

The bedrock consists mostly of Nubra-Siachen batholith and leucogranites (Phillips, 2008). In a wetter and stronly glaciated Karakoram, the cushions descend to lower elevations compare to dry Little Tibet where the cushions ascend to 5900 m due to a poor glaciation and relatively well developed soils at high elevations (Dvorský et al., 2011). Zonation of the vegetation is similar to the Little Tibet, but the vegetation zones are shifted downwards by 300–400 metres because of higher elevation of surrounding mountains and their large-scale glaciation leading to development of rather extensive alpine grasslands around glacial lakes and morianes at 5000–5200 m.

The second locality is the valley of the Lupgo stream on the western side of Chamser Kangri Peak, near Tso Moriri Lake; this belongs to Changtang region in the eastern part of Ladakh (32°59´N, 78°24´E). This area of the Trans-Himalaya can be characterised as a high-elevation plain, being the westernmost spur of the Tibetan Plateau (also called Little Tibet). It is of relatively flat relief with gentle slopes bearing many rounded and poorly glaciated peaks, reaching 6666 m (Lungser Kangri). The substrate ranges from siliceous rocks (Precambrian granites, Tso Moriri gneiss) to calcareous and saline sediments (Phillips, 2008). At lower elevations much of the area is covered by cold desert and semi-desert vegetation, a steppe zone is found at middle elevations up to c. 5000–5400 m, alpine grasslands form a narrow belt above the steppe zone and subnival vegetation is developed at the highest elevations of 5700–6000 m (Klimeš and Doležal, 2010; Dvorský et al., 2011). The snow line, in its climatological sense (Zingg, 1954), is situated at 6100–6200 m, i.e. coinciding with the greatest recorded elevation of vascular plants in the region (c. 6150 m).

Target species

The target species of this study, *T. caespitosum*, is one of the most prominent high-alpine cushion plants in the Himalayas. It is a perennial plant with a woody taproot and it forms very dense and solid cushions (Klimešová et al. 2011). Although very little is known about its speed of growth or longevity, the largest cushions in the study region can be more than 150 cm in diameter and may persist for decades or even centuries (Kleier and Rundel, 2004; Le Roux and McGeoch, 2004). In the study region, *T. caespitosum* occurs in the elevational range of 4600–5900 m (Dvorský et al., 2011; Klimešová et al., 2011). In Nubra, *T. caespitosum* can be found from 4600 to 5480 m, from dry alpine steppes at lower elevations (with poorly developed sandy soils dominated by *Tanacetum tibeticum*, *Artemisia minor* and *Elymus schrenkianus*) to rocky outcrops at higher elevations surrounded by glacier moraines and mesic alpine meadows (dominated by *Potentilla pamirica*, *Poa attenuata*, and *Astragalus confertus*). In the Tso Moriri locality, the cushions occur from 5100 to 5960 m, from dry alpine screes at lower elevations (dominated by *Poa attenuata*, *Urtica hyperborea* and *Dracocephalum heterophyllum*), to the subnival zone with poorly developed soils covered by algal crusts and few vascular plants (*Saussurea gnaphalodes*, *Draba altaica* and *Stellaria decumbens*). Plant scientific names follow Klimeš and Dickoré (2006).

Vegetation sampling

At both localities, 4 sites along an elevational gradient were selected to cover the entire elevation range of *T. caespitosum*: in Nubra Valley at 4850 m, 5000 m, 5100 m and 5250 m and in Tso Moriri at 5350 m, 5600 m, 5750 m and 5850 m. At each elevational site, we systematically surveyed cushions within an area of ca. 1 ha so that we received sufficient replications (in Nubra n = 66, 61, 69, 77; in Tso Moriri n = 70, 70, 70, 73). The surveyed cushions represented all size classes with a diameter range of 4–132 cm. However, the most common size class was 40–60 cm in Nubra (n = 79) and 20–40 cm in Tso Moriri (n = 102). Two vegetation samples were taken at each cushion: one covering the cushion itself, the other one had the same size and shape and was marked with a flexible wire ring which was placed randomly in the open site outside the cushion at a distance equalling the cushion diameter. If the spot was occupied by another cushion or a stone covering the whole area of the sample, the sample was taken on the opposite side of the cushion. We recorded vascular plant species rooting within the respective sample areas and their percentage cover. At all sites, *T. caespitosum* was one of the few dominant species (Dvorský et al., 2011). Its relative cover increased with increasing elevation (from 5 to 15%, based on data from 44 square plots 100 m^2 in size, sampled between 4700–5850 m, Dvorský et al., 2011; Klimešová et al., 2011). The total vegetation cover ranged from 10 to 40%. The fieldwork was carried out during the peak of the vegetation season (August) in 2009–2011. No specific permits were required for the described field studies, the locations were not privately-owned or protected in any way and the field studies did not involve endangered or protected species.

Microclimatic measurements

We recorded air temperature and relative air humidity using datalogger HOBO U23 Pro v2 placed 10 cm above the soil surface and shielded against direct sunlight. The measurements were recorded every two hours from August 2009 to August 2011. Additionally, at three sites (Nubra 5000 m, Tso Moriri 5600 m and 5850 m), we chose one cushion of average size and placed a temperature logger (iButton® DS1923, Maxim Integrated Products) in the soil below it and another one in the soil of the adjacent open area 50 cm from the cushion. Inside the cushions, the loggers were placed 2 cm deep in the substrate under the cushion tissue, where the colonising species were thought to be rooting. In the open areas, the loggers were burried 2 cm under the soil surface. The measurements were recorded every three hours from September 2009 to August 2010.

Soil sampling

During August 2009, we collected soil samples for supplementary physico-chemical analyses from 6 cushions within the most common size class at each elevational site (96 samples in total). We took one soil sample (150 g) below a cushion and one sample in the open site. The samples were air-dried for 10 h on an aluminium plate, placed in sterile 540 ml polypropylene bags (Nasco Whirl-Pak®). In the laboratory, the samples were weighted, oven-dried at 100 °C, ground in a mortar and sieved to 2 mm fraction after the removal of roots. The analysed components included total nitrogen (TN), NH_4^+-N, NO_3–N, PO_4^{-3}-P, Ca^{2+}, Mg^{2+}, Na^+, K^+, pH, texture (percentage content of particles larger than 0.5 mm), gravimetric water content (GWC) and organic matter (OM). Soil physico-chemical analyses were conducted in accordance with the standardised methods of the Association of German Agricultural Analytical and Research Institutes (VDLUFA 1991). Soil pH was potentiometrically measured in a suspension with 0.01 M $CaCl_2$. Plant available N and P content of the soil samples was analysed colorimetrically with an FIAstar 5010 Analyzer (Foss Tecator AB, Höganäs, Sweden) and the cations with AAS (SpectrAA 640, Varian Techtron, Melbourne, Australia). Soil moisture was futher measured at each soil sampling point as percent volumetric water content (VWC) using Hydro-Sense Measurement System (Campbell Scientific, Australia).

Data analyses

In all statistical analyses, the two localities (Nubra and Tso Moriri) were treated separately as they represent a separate gradient going from a cold steppe zone to a subnival zone. Further in the text, we distinguish two vascular plant habitats: 1) inside cushions and 2) open areas outside cushions.

To assess whether the species richness of vascular plants associated with cushions differed from those in open areas, we calculated sample-based rarefaction curves (a measure of total species richness or species pool) (Gotelli and Colwell, 2001) separately for each habitat and elevation site. The curves were computed as means of 9999 sample-based species accumulation curves that resulted from the random ordering of all plots belonging to each habitat type. The calculation was performed with the Juice program (Tichý, 2002).

To assess whether the number of species increases with the sample area (i.e. cushion size) and to compare these species-area relationships between cushions and open areas, we fitted a multiple linear model with log(species richness) as the dependent variable, log(sample size) as the continuous predictor and the sample position as the categorical predictor variable. This was done for each study site. We additionally performed a test for differences in slope and intercept parameter estimates between the two regression lines as implemented in the *smatr* package (Warton, 2009) for R software (R Development Core Team, 2010). The significant deviation from the common intercept indicated differences in species richness per unit area between cushions and open areas. The contrasting slopes (*test of parallelism*) indicated a significant difference in the rate at which species richness increased with enlarging area.

To explore whether species composition of vascular plants growing on cushions differed from those in open areas, we performed a non-metric multidimensional scaling (NMDS) on a Bray-Curtis dissimilarity matrix, which was calculated from square-root transformed percentage cover data standardised by totals of samples. We ran NMDS in two dimensions and used several random starts in order to achieve the optimum configuration. The results were visualised by an ordination diagram with 95% confidence ellipses around multivariate centroid of samples from each habitat type. We further used permutational multivariate analysis of variance (PERMANOVA) to test the differences in species composition between the cushion habitat and open areas (Anderson, 2001). We assessed the significance of the cushion effect by a nonparametric test with 999 permutations restricted within the blocks (i.e. a pair of neighboring cushion/non-cushion samples). NMDS and PERMANOVA were calculated separately for each elevation site using *meta*MDS and *adonis* functions from vegan library for R software (Oksanen et al., 2011).

To assess whether individual plant species were significantly more associated with cushions or with open areas, we used the Friedman test, based on 9999 Monte-Carlo resamplings (Hollander and Wolfe, 1999). This is a nonparametric test assessing the symmetry of responses across treatments for repeated measures or split-plot data. The two positions (i.e. within and outside cushions) were considered as repeat measures for each individual cushion. The Friedman test was applied to presence/absence as well as abundance (percent cover) data in each species. We analysed the degree of association of each species with cushions for each elevation site to evaluate whether there is an increasing number of facilitated species with elevation. Analyses were run using the *coin* package (Hothorn et al., 2008) for R software.

In order to quantify the outcome of interactions between *Thylacospermum* and other species, we calculated two widely used plant-plant interaction indices: (1) RII - relative interaction intensity index (Armas et al., 2004), and (2) I_{imp} - relative importance index (Seifan et al., 2010). These are reffered to as competitive intensity and importance and the index values range from competition (-1) to facilitation (+1) and are symmetrical around 0. They can also be scaled up to measure interactions at both individual and community levels within each elevation site. We calculated RII and I_{imp} values at the community level by pooling individual values of all species in vegetation samples. As for individual species both indices yielded similar results, we present only the RII values (see Fig. S1).

In order to reveal differences in the soil physico-chemical parameters between cushions and open areas and their dependence on elevation, we used linear mixed-effect models or generalised linear mixed-effect models, depending on the nature of a particular response variable (assuming Gaussian, quasi-Poisson, or quasi-binomial distributions). The pair-samples represented a random effect factor, and elevation and position (cushions and open areas) were fixed effect factors. The tests were based on the likelihood-ratio approach, approximating the difference in model deviances with a χ^2 distribution. To control for familywise error rate, the false discovery rate procedure was performed (Benjamini and Hochberg, 1995). Analyses were run using the *lme4* package (Bates and Maechler, 2010) in R software.

Results

Species richness

In total, 56 species were recorded (39 in Nubra, 30 in Tso Moriri, Tables 2 and 3), belonging to 15 families. The richest family at both localities was Asteraceae (nine species in Nubra, six in Tso Moriri). Fourteen species (25%) were common for both localities.The total percentage cover of plant species growing on bare ground outside cushions was significantly higher than that of species established inside cushions (Nubra: 30.4 vs 10.9%, Tso Moriri: 10.6 vs 1.8%). The total cover outside cushions decreased significantly with increasing elevation at both localities (Nubra: from 41 to 23% between 4850–5250 m, Tso Moriri: from 14 to 8% between 5350–5850 m), while non-significant elevation differences were found for the values inside cushions (Fig. 3).

The maximum number of species recorded within a single cushion or open area was 8 and 9, respectively. In all elevation sites, species richness increased with the sample area, both inside cushions and in the surrounding open areas (Fig. 4). However, the intercepts of regression lines for cushions were always significantly smaller than those obtained for the open areas (Table S1), showing that an average open area at all sites always harboured more species than an average cushion. Across the entire elevation range of *T. caespitosum*, the numbers of species were significantly higher outside cushions (Fig. 4) with the exception of Nubra 5250 m, where there were nonsignificant diferences in species richness (Table S1). Slopes of regression lines showed no difference between the two habitats in all study sites, indicating that the difference in species richness between the cushions and open areas were constant across the sample sizes.

The total species richness at the maximal number of samples, estimated from the sample-based rarefaction curves (Fig. 5), followed the same pattern as the local richness, with fewer species inside the cushion. However, the magnitude of these differences varied among the study sites. In Nubra, the differences between the two habitats were small (on average 8.1% more species in the open areas than cushions), with the exception of the lowest (driest) site in 4850 m (36% more species in the open areas). In Tso Moriri, open areas in all four elevation sites contained significantly more species than cushions (on average 30% more species in the open areas).

Vegetation composition and habitat preferences of individual species

In all study sites, the species composition of vascular plants growing on cushions differed significantly from that in open areas (Table S2), although the magnitude of compositional dissimilarity also varied between the sites. This was indicated by different degrees of overlap between the confidence intervals of centroids for cushions and open areas in the NMDS ordination diagrams (Fig. 6). In Nubra, 15 species (39.5%) showed a preference for a habitat and all of these species preferred open areas (Table 2). In the case of *Poa attenuata*, the preference was consistent over all four elevational sites. *Astragalus strictus* and *A. confertus* preferred open areas in all of the three sites they occurred in. *Potentilla pamirica* preferred open areas at the three lower sites but not at the highest site. In Tso Moriri, 18 species (60%) showed a preference and all of these species again preferred open areas (Table 3). *Poa attenuata*, *Draba altaica*, *Eritrichium hemisphaericum* and *Saussurea gnaphalodes* preferred open areas at three sites. The least number of species having a significant habitat preference for areas outside cushions was found in Nubra 5250 m, while the highest numbers of species prefering open areas occurred in the two subnival sites in Tso Moriri 5700–5850 m, and in the steppe site in Nubra 5000 m. There were species with the same occurrence pattern but different statistical significance of habitat preference; this was because of differences in cover-abundance data between the cushion and the outside plot.

Table 2. Species frequency of occurrence inside (in) and outside (out) cushions of *T. caespitosum* at four elevation sites in Nubra. Friedman-based resampling on presence/absence (Fp) and cover-abundance (Fa) data was used to define the preferred position of a species (*** P < 0.001, ** P < 0.01,* P < 0.05). All significant preferences refer to habitat outside cushion.

Species	4850 m				5000 m				5100 m				5250 m			
	in	out	Fp	Fa	in	out	Fp	Fa	in	out	Fp	Fa	in	out	Fp	Fa
Poa attenuata	34	50	***	***	24	39	***	***	19	30		**	30	41		**
Potentilla pamirica	0	8	**	*	14	28	**	**	18	26		***	31	29		
Oxytropis tatarica	0	0			1	5			23	32		*	20	32	*	*
Astragalus strictus	30	45	**	***	11	23	*	**	0	1	***	***	0	0		
Astragalus confertus	0	0			6	12		*	15	25		*	19	24		*
Draba altaica	0	2			3	18	***	***	10	16			22	30		
Leontopodium leontopodinum	19	21			7	18	**	***	4	2			4	4		
Oxytropis densa	34	34			5	6			0	0			0	0		
Artemisia minor	12	37	***	***	11	16			0	0			0	0		
Carex pseudofoetida	10	13		**	9	8			1	2			10	10		
Tanacetum tibeticum	5	9			4	11		*	5	15	*	*	4	5		
Potentilla bifurca	15	21			4	4			0	0			0	0		
Lloydia serotina	0	0			4	3			0	1			18	15		
Elymus schrenkianus	8	7			0	0			5	4			1	0		
Potentilla gelida	0	0			6	18	***	***	0	0			0	0		
Potentilla multifida agg.	4	10			1	2			0	0			0	0		
Oxytropis platysema	0	0			7	7			0	0			1	1		
Saussurea glacialis	0	0			1	0			4	1			5	5		
Taraxacum sp.	0	0			3	10	*		0	0			1	1		
Trisetum spicatum	0	0			6	6			0	0			1	0		
Oxytropis chiliophylla	0	0			3	8	*	*	0	0			0	0		
Carex sp.	5	4			0	0			0	0			0	0		
Gentianella azurea	1	4			0	0			0	0			0	4		
Artemisia santolinifolia	0	8	***	***	0	0			0	0			0	0		
Elymus schugnanicus	3	4			0	0			0	0			0	0		
Braya humilis	0	0			1	4			0	0			0	1		
Thalictrum alpinum	0	0			1	1			0	0			1	3		
Aster flaccidus	0	0			0	0			0	0			1	2		
Ephedra gerardiana	2	1			0	0			0	0			0	0		
Oxytropis pusilla	0	2			0	0			0	0			0	1		
Saussurea gnaphalodes	0	0			0	0			2	0			1	0		
Urtica hyperborea	0	2		***	1	0			0	0			0	0		
Potentilla saundersiana	0	2			0	0			0	0			0	0		
Waldheimia tridactylites	0	0			1	1			0	0			0	0		
Carex borii	0	0			0	0			0	0			0	1		
Kobresia schoenoides	0	0			0	0			0	0			1	0		
Lomatogonium thomsonii	0	1			0	0			0	0			0	0		
Sibbaldia tetrandra	0	0			0	0			0	0			0	1		
total	182	285	5	7	134	248	8	9	106	155	2	6	171	210	1	3
average richness	3	4			2	4			2	2			2	3		
total richness	14	21			24	22			11	12			18	19		

Outcome of plant–plant interactions

The RII and I_{imp} indices calculated at community level revealed that the effects of *T. caespitosum* cushions on neighbouring plants were significantly negative at all elevations at both Nubra and Tso Moriri (Fig. 7). This suggests the prevalence of competitive interactions. RII values were significantly lower in Tso Moriri when compared with Nubra, indicating more intense competition in more

Table 3. Species frequency of occurrence inside (in) and outside (out) cushions of *T. caespitosum* at four elevation sites in Tso Moriri (see Table 2 for explanation).

Species	**5350 m**				**5600 m**				**5750 m**				**5850 m**			
	in	**out**	**Fp**	**Fa**	**in**	**out**	**Fp**	**Fa**	**in**	**out**	**Fp**	**Fa**	**in**	**out**	**Fp**	**Fa**
Poa attenuata	15	26	**		34	46	*	**	43	49		***	27	43	**	**
Draba altaica	0	0			20	39	**	**	32	64	***	***	29	50	***	***
Saussurea gnaphalodes	1	2			12	20	*	***	16	29	**	**	24	38	*	*
Desideria pumila	0	0			3	7			22	21			10	13		
Stellaria decumbens	0	0			0	3			0	28	***	***	0	26	***	***
Artemisia minor	17	37	***	**	0	0			0	0			0	0		
Waldheimia tridactylites	0	0			6	17	**	**	0	0			8	13		*
Eritrichium hemisphaericum	0	0			0	10	***	***	0	9	**	**	1	15	**	***
Arenaria bryophylla	2	5			5	15	**	**	0	4			0	0		
Alyssum klimesii	0	0			2	1			1	1			7	15	*	*
Saussurea glacialis	0	1			0	0			6	17	*		1	2		
Potentilla pamirica	1	4			5	11			0	4	***	***	0	1		
Dracocephalum heterophyllum	10	11			0	0			0	0			0	0		
Carex sagaensis	2	3			0	2			4	8		**	0	0		
Oxytropis chiliophylla	2	17	***	***	0	0			0	0			0	0		
Saxifraga nanella	0	0			0	0			0	0			3	16	***	***
Stellaria depressa	5	10		***	0	3			0	0			0	0		
Saussurea hypsipeta	0	0			0	0			2	11	*	**	0	0		
Draba oreades	2	7			0	0			0	0			1	0		
Elymus schrenkianus	2	7		*	0	0			0	0			0	0		
Aphragmus oxycarpus	0	0			0	0			1	3			1	3		
Astragalus confertus	0	0			0	8	***	***	0	0			0	0		
Elymus schugnanicus	3	4			0	0			0	0			0	0		
Delphinium brunonianum	0	5			0	0			0	0			0	0		
Urtica hyperborea	0	4			0	0			0	0			0	0		
Festuca tibetica	0	0			0	0			2	0			0	0		
Saxifraga cernua	0	0			0	0			0	0			0	2		
Carex sp.	0	0			1	0			0	0			0	0		
Saussurea glanduligera	0	1			0	0			0	0			0	0		
Stipa subsessiliflora	0	1			0	0			0	0			0	0		
total	62	145	3	4	88	182	7	7	129	248	7	8	112	237	7	8
average richness	0.9	2.2			1.3	2.8			1.8	3.7			1.5	3.3		
total richness	12	17			9	13			10	13			11	13		

extreme elevations. In both localities, however, there was a tendency for competitive intensity and importance to diminish with increasing elevation (less negative values towards higher elevations; Fig. 7). Species-specific responses to the presence of cushions were negative in 78% of tested relationships (19 positive responses out of 86 analyzed in total) in Nubra, and 92% in Tso Moriri

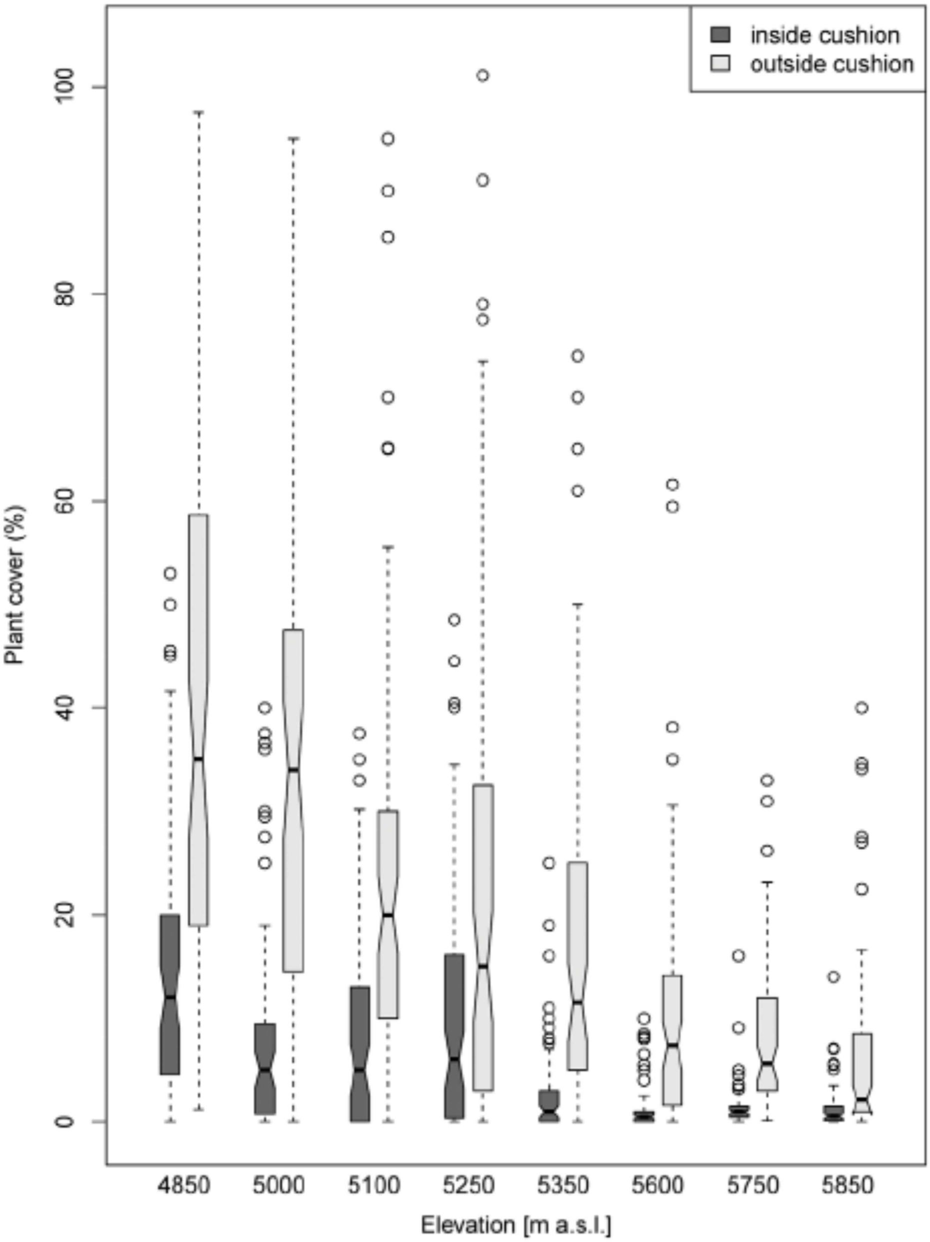


Fig. 3. Comparision of total cover of vascular plants growing on the *Thylacospermum caespitosum* cushions and open areas outside cushions (vegetated area, the rest is bare soil or soil crusts) in Nubra (4850–5250 m) and Tso Moriri (5350–5850 m). The surveyed cushions represented all size classes with a diameter range of 4–132 cm. Boxes represent 25–75% of values, black dots are medians, whiskers are 1.5 interquartile ranges and open dots are outliers. Notches in the boxes indicate the significance of between-group differences: if notches of two groups do not overlap this is strong evidence that these groups differ significantly.

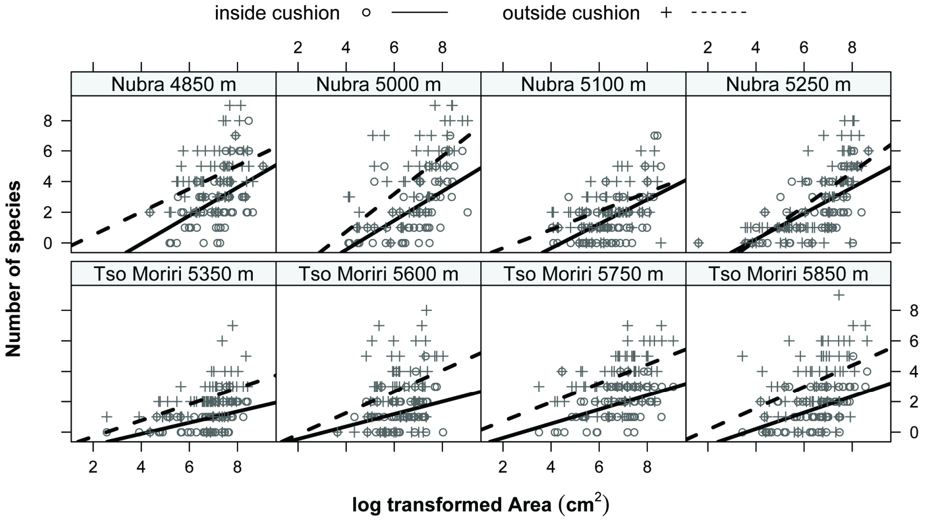


Fig. 4. Species-area relationships for cushion and open areas on each study site. Statistics for each regression analysis are provided in Table S1.

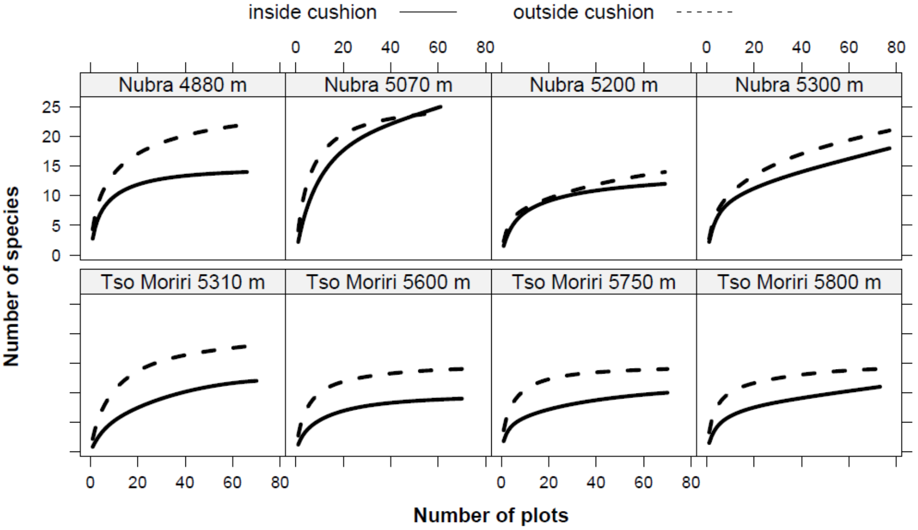


Fig. 5. Sample-based rarefaction curves for cushions and open areas on each study site. Note an increase in the number of species encountered with increasing number of plots sampled. Open areas outside cushions are more species rich than equal areas inside cushions at all elevations.

(5 positive interactions out of 63 in total; Fig. S1). Most species with positive RII and I_{imp} values had a low (<5 records) frequency of occurrence (Table 2 and 3), and none of them showed a significant habitat preference in the Friedman-based permutation test.

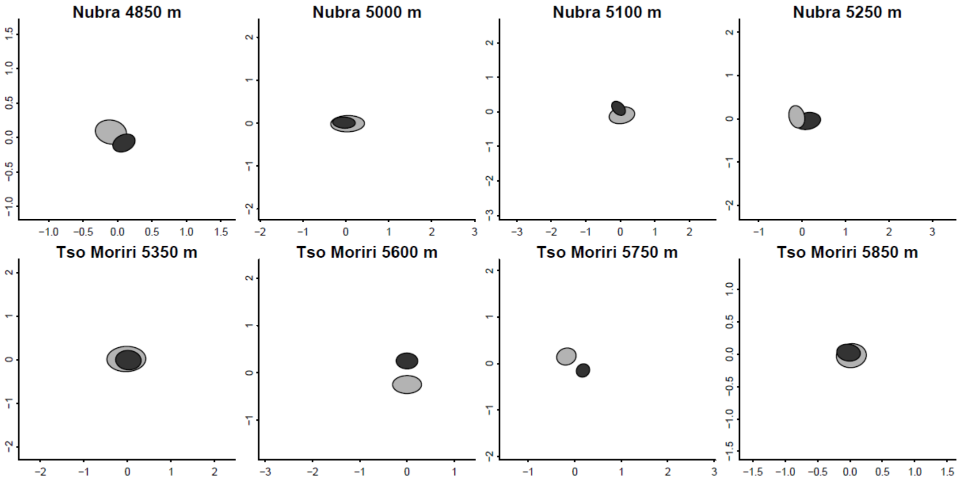


Fig. 6. Differences in species composition of vascular plants between the cushions (black) and open areas outside cushions. Diagrams are from non-metric multidimesional scaling (NMDS). Each diagram shows 95% confidence ellipses around multivariate centroid of samples from cushions (black) and open areas outside cushions (grey). Differences are statistically significant (p,0.01) at all elevations (detailed results from PERMANOVA are given in Table S2).

Microclimate

In Nubra the vegetation season, defined as the period with mean daily temperatures above freezing, lasted between three months (mid May to beginning of September) at 5250 m and five months (the beginning of May to mid October) at 4850 m. The mean air annual/summer temperatures decreased from -1.6/ 7.7 °C to -3.6/7.1 and the relative air humidity increased from 39/38% to 87/69% with increasing elevation. At all sites, air temperatures in the warmest month of August remained above zero both day and night. Compared with the similar elevation in Tso Moriri, a wetter Nubra site at 5250 m had ~ two months shorter growing season due to deeper snowpack which persisted longer into the spring (personal observation). This leads to a higher water supply and the development of alpine grasslands compared to the dry steppes in Tso Moriri. This was also evident from differences in winter temperatures, which were higher and less fluctuating in Nubra than in Tso Moriri due to more effective snow protection (mean ± S.D.: -7.9±0.7 °C vs -16.8±3.1 °C during January–February).

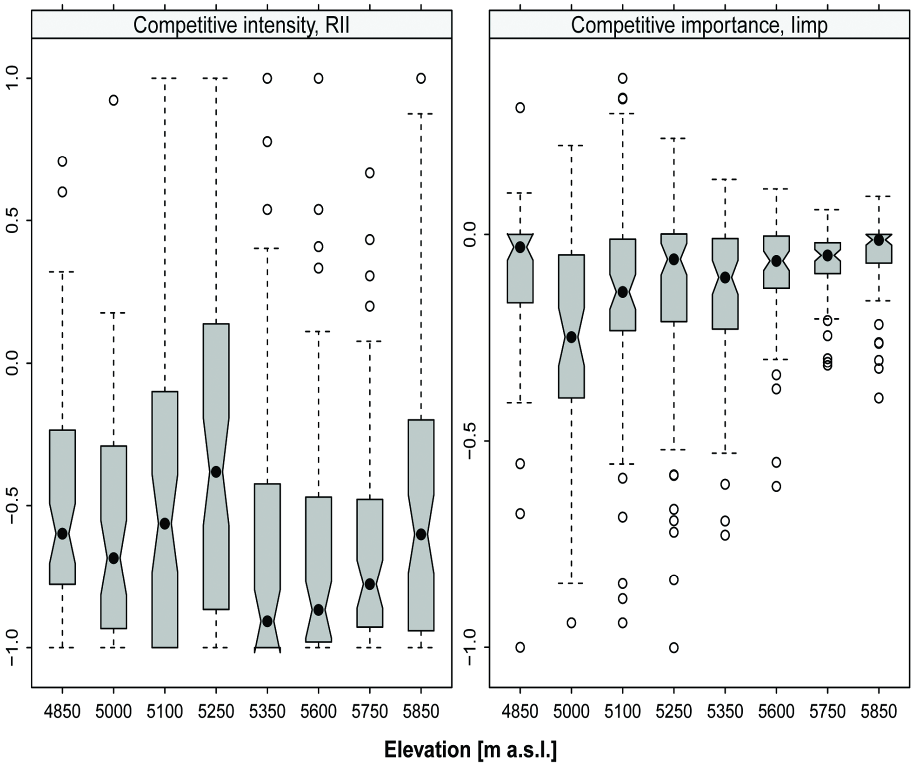


Fig. 7. Outcome of plant–plant interactions at community level on each study site in Nubra (4850–5250 m) and Tso Moriri (5350–5850 m). Shown are the competitive intensity and competitive importance values between *T. caespitosum* and other species. Responses were calculated such that competition is represented by negative values and facilitation by positive values. Individual species responses to the presence of cushions are given in Fig. S1.

In Tso Moriri, the vegetation season lasted nearly five months at 5350 m, 3.5 months at 5600 m, and three months at 5750 m. At the highest elevation, the vegetation season was restricted to less then two months (Table 3). Even during August, when temperatures generally reached their highest values, temperatures regularly dropped below freezing during the night; at the highest sites repeatedly to about -5 °C. The sites differed mainly in the duration of the sub-zero temperature spells over the course of a 24-hour period. While air temperature 10 cm above ground never dropped below zero at the lowest elevation during August, it usually fell below zero for about 2–3 hours at the middle elevations; at the highest elevation at 5850 m freezing lasted between 5 and 10 hours every day, particularly in the second half of August when many plants still flowered and fruited. Daily air temperatures rose to 15–20 °C at all four elevations but for a much shorter time per day at higher elevations. The mean air annual/summer temperatures decreased from -4.4/7.3 °C and -10.4/4.4 °C between 5350–5850 m, while relative air humidity increased from 61/50% to 84/53%.

Table 4. Soil temperature regime and degree days (°d) at 2–3 cm depth inside and outside cushions. Measured in 3-hour intervals from 1.9.2009 to 31.8.2010. Values with the lowercase superscript letter indicate situation when there was even an earlier day with $T_{mean}>0$ on the beginning of the vegetation season (a - 2.6., b - 28.5., c - 6.5., d - 8.6.); these early days however were often followed by a significant period of days with Tmean<0, that is why a later date is used in the table so that it better represents the beginning of the vegetation season. During the frost-free period no freezing temperatures were recorded, even if mild soil freezing might have occurred.

		Nubra 5000 m		**Tso Moriri 5600 m**		**Tso Moriri 5850 m**	
	Temperature [°C]	in	out	in	out	in	out
Whole year	T_{mean}	-0.3	-3.4	-6.7	-6.4	-7.4	-7.4
	T_{max}	20.2	16.2	19.2	15.2	16.2	17.2
	T_{min}	-15.6	-15.5	-22.1	-20.5	-25.6	-26.1
	daily $T_{mean}>0$ °C	8.4.–14.10.	5.5.–10.10.	5.6.–27.9.[a]	2.6.–5.10.[b]	20.6.–27.9.[c]	20.6.–24.9.[d]
	frost-free period	9.4.–10.10.	5.5.–9.10.	4.7.–25.8.	20.6.–4.9.	21.6.–3.9.	3.7.–2.9.
	°d (T_{base}=0 °C)	1227	601	434	391	330	351
	°d (T_{base} =3 °C)	736	329	196	162	123	137
Growing season (15.V.–15.IX.)	T_{mean}	7.7	4.0	3.1	2.7	2.1	2.3
	T_{max}	20.2	16.2	19.2	15.2	16.2	17.2
	T_{min}	0.6	0.1	-10.0	-7.9	-8.9	-11.0
	°d ($T_{base}>0$ °C)	949	500	427	385	326	349
	°d ($T_{base}>3$ °C)	600	294	196	162	123	137

Cushions from a low site in Nubra (5000 m) provided warmer microsites, as measured 2 cm below ground, compared with open areas (annual mean T = -0.3 °C vs. -3.4 °C). They also had twice as many degree-days (1227 vs. 601 at T_{base}= 0 °C) and a frost-free period lasting a month longer (Table 3). On the other hand, the differences at the highest site in Tso Moriri were only minor, the open areas being even slightly warmer than cushions (Table 4, Fig. 8).

Soil

The contents of NH_4^+-N, PO_4^{-3}-P values were higher in the soil of open areas at both localities (Table 5). PO_4^{-3}-P and K content significantly decreased with elevation while the Na content increased with elevation at both localities. In Nubra only, more Mg was found in the soil of open areas, while the OM content was higher in the soil below cushions, significant at 5000 and 5100 m elevations. The OM and concentration of NH_4^+-N decreased with elevation. Some

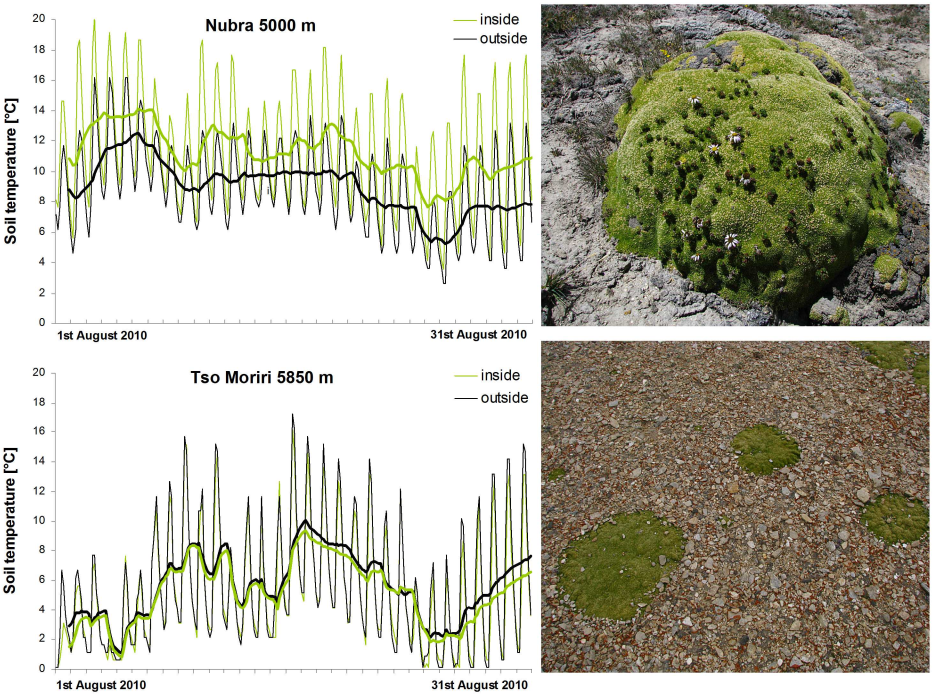


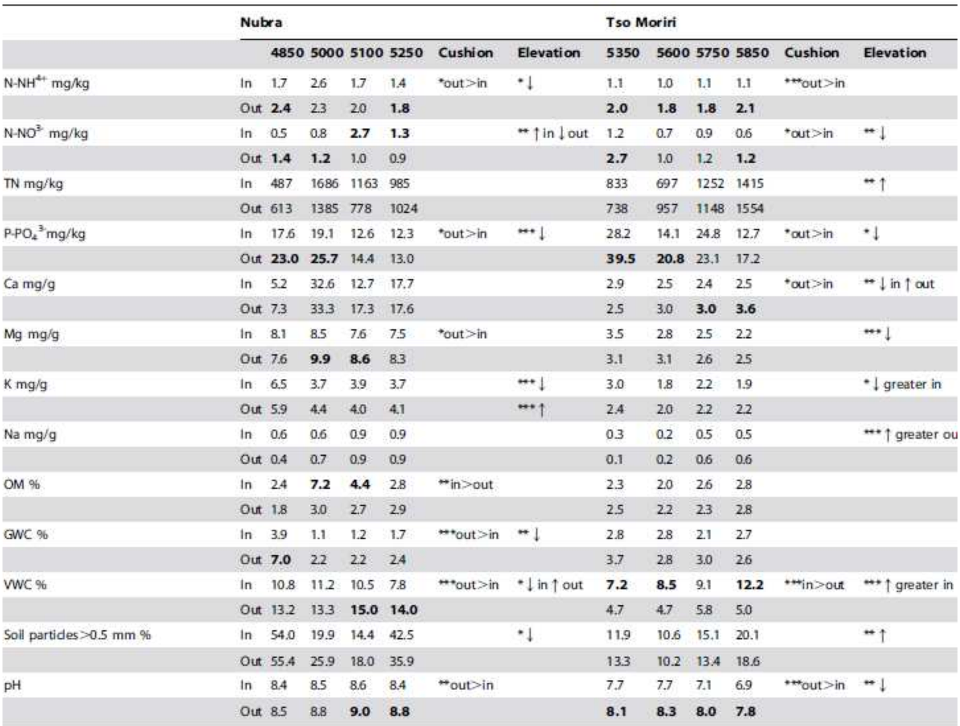
Fig. 8. Soil temperature regime. Sampled inside the *T. caespitosum* cushion and in the soil of the adjacent open area at Nubra 5000 m and Tso Moriri 5850 m. Thick lines depict the moving average for eight daily measurements. Photos on the right side of the panel show the variability in the growth of cushions; generally, individuals from lower elevations are gibbous and protruding above the ground, individuals from the upper part of the distributional gradient are flat.

interactions were revealed: the NO_3^--N content increased inside the cushions and decreased in the open areas with increasing elevation. Soil water content was low at both localities, from 2 to 17%; the VWC in Nubra was higher outside the cushions, significantly at 5100 and 5250 m elevations, and increased in the open areas and decreased inside the cushions with increasing elevation.

In Tso Moriri only, NO_3^--N and Ca contents were higher in the soil of open areas, most significantly at 5350 and 5850 m elevations. Considering the trends with elevation irrespective of the habitat, the contents of NO_3^--N and Mg in the soil decreased, while the total N increased (Table 5). Some interactions were also revealed: the Ca content in the soil decreased with elevation inside the cushions and increased in the open areas. The pH and K content in the soil decreased with elevation. This decrease was greater inside the cushions. The

VWC in Tso Moriri was higher inside the cushions and this difference increased with elevation. The Na content increased with elevation and this increase was greater in the open areas.

Table 5. Soil physico-chemical characteristics. Availability of soil nutrients in the presence (in) or absence (out) of *T. caespitosum* cushions. An upward or downward pointing arrow indicates a positive or negative relationship between the dependent variable and elevation, based on the likelihood-ratio test (*** P<0.001, ** P<0.01, * P<0.05). Post hoc Tukey tests are shown on paired differences (significantly higher values inside/outside cushion are in bold).



		Nubra						**Tso Moriri**					
		4850	**5000**	**5100**	**5250**	**Cushion**	**Elevation**	**5350**	**5600**	**5750**	**5850**	**Cushion**	**Elevation**
N-NH^{4+} mg/kg	In	1.7	2.6	1.7	1.4	*out>in	* ↓	1.1	1.0	1.1	1.1	***out>in	
	Out	**2.4**	2.3	2.0	**1.8**			**2.0**	**1.8**	**1.8**	**2.1**		
N-NO^{3-} mg/kg	In	0.5	0.8	**2.7**	**1.3**		** ↑ in ↓ out	1.2	0.7	0.9	0.6	*out>in	** ↓
	Out	**1.4**	**1.2**	1.0	0.9			**2.7**	1.0	1.2	**1.2**		
TN mg/kg	In	487	1686	1163	985			833	697	1252	1415		** ↑
	Out	613	1385	778	1024			738	957	1148	1554		
P-PO_4^{3}mg/kg	In	17.6	19.1	12.6	12.3	*out>in	*** ↓	28.2	14.1	24.8	12.7	*out>in	* ↓
	Out	**23.0**	**25.7**	14.4	13.0			**39.5**	**20.8**	23.1	17.2		
Ca mg/g	In	5.2	32.6	12.7	17.7			2.9	2.5	2.4	2.5	*out>in	** ↓ in ↑ out
	Out	7.3	33.3	17.3	17.6			2.5	3.0	**3.0**	**3.6**		
Mg mg/g	In	8.1	8.5	7.6	7.5	*out>in		3.5	2.8	2.5	2.2		*** ↓
	Out	7.6	**9.9**	**8.6**	8.3			3.1	3.1	2.6	2.5		
K mg/g	In	6.5	3.7	3.9	3.7		*** ↓	3.0	1.8	2.2	1.9		* ↓ greater in
	Out	5.9	4.4	4.0	4.1		*** ↑	2.4	2.0	2.2	2.2		
Na mg/g	In	0.6	0.6	0.9	0.9			0.3	0.2	0.5	0.5		*** ↑ greater ou
	Out	0.4	0.7	0.9	0.9			0.1	0.2	0.6	0.6		
OM %	In	2.4	**7.2**	**4.4**	2.8	**in>out		2.3	2.0	2.6	2.8		
	Out	1.8	3.0	2.7	2.9			2.5	2.2	2.3	2.8		
GWC %	In	3.9	1.1	1.2	1.7	***out>in	** ↓	2.8	2.8	2.1	2.7		
	Out	**7.0**	2.2	2.2	2.4			3.7	2.8	3.0	2.6		
VWC %	In	10.8	11.2	10.5	7.8	***out>in	* ↓ in ↑ out	**7.2**	**8.5**	9.1	**12.2**	***in>out	*** ↑ greater in
	Out	13.2	13.3	**15.0**	**14.0**			4.7	4.7	5.8	5.0		
Soil particles>0.5 mm %	In	54.0	19.9	14.4	42.5		* ↓	11.9	10.6	15.1	20.1		** ↑
	Out	55.4	25.9	18.0	35.9			13.3	10.2	13.4	18.6		
pH	In	8.4	8.5	8.6	8.4	**out>in		7.7	7.7	7.1	6.9	***out>in	** ↓
	Out	8.5	8.8	**9.0**	**8.8**			**8.1**	**8.3**	**8.0**	**7.8**		

Discussion

It is generally assumed that the diversity and composition of plant communities is determined by a species pool (i.e. a set of species propagules which are able to reach the site), an environmental filter (environmental restrictions on species survival) and a community filter (biotic interactions, mainly competition) (Lepš, 2005). The species pool size is correlated with the surrounding area of a given habitat (Romdal and Grytnes, 2007) and hence its effect on local species richness is relatively straightforward. The effect of the community filter is probably the least predictable one – each species is unique. The presence of a strong competitor, which is able to dominate the community and have an impact on other species, is difficult to predict and may have historical and/or environmental reasons. SGH, in particular, predicts that strong competitors will occur with higher probability in favourable environments having ample resources. These are less likely in stressful environments, e.g. at higher elevations, where neutral interactions or even facilitation by dominant plants may prevail over competition (Maestre et al., 2005).

We investigated the association pattern of plants with the dominant cushion plant *T. caespitosum* and assessed its influence on microclimate and soil physico-chemical properties in communities along an elevational gradient in a dry region of the Indian Trans-Himalaya. Our hypotheses were based on the fact that cushions are not only well adapted to the harsh climate of high mountains but, owing to their specific characteristics, are also able to positively modify microsite conditions. This helps other species establish, survive or perform better. There are about two dozen studies focusing on this topic, with practically all of them supporting this idea (Table 1). With just a few exceptions (de Bello et al., 2011), cushion plants were proven to facilitate other plant species and enhance the local plant richness and diversity. The facilitation was more prominent in more stressful environments, such as relatively high elevations, indicating the validity of SGH. Our study, however, shows something rather different. We attribute this to a combination of extreme elevation and aridity in our study area: cushions do not provide better microsites for other plants under these conditions.

Microclimate and soil nutrients

In lower elevations, cushions increased soil temperature and prolonged the growing season in comparison with the adjacent open areas. These differences, however, diminished with elevation such that the open substrates even tended to be warmer at the highest elevations; this was also observed by Cavieres et al. (2007). Badano et al. (2006) noticed that cushions of *Azorella monantha* maintained a lower temperature than the open area for much of the time, as was the case at our uppermost site. At Nubra (5000 m) and Tso Moriri (5600 m), there was a wider variation in temperature within cushions than in the open area while we expected the opposite to be true (Cavieres et al., 2006).

The nursing effect of cushions, besides the other mechanisms mentioned above, is often attributed to better nutrient availability in the soil below them (Nuñez et al., 1999; Cavieres et al., 2006, 2008; Yang et al., 2010). Our results contradict these findings. Soil the PO_4^{-3}-P and NH_4^+-N contents were lower in the soil below cushions at both localities. Moreover, K decreased with elevation and the decrease was greater below the cushions. Additionally, the soils below cushions contained less Mg in Nubra and less NO_3-N and Ca in Tso Moriri. Only the higher content of OM in soils below cushions (in Nubra only) was consistent with earlier reports (Nuñez et al., 1999). The markedly high contents of Mg and K in soils from Nubra are caused by the specific geochemistry of the Karakorum batholith (rich in Mg) and leucogranites (rich in K) (Phillips, 2008). The main source of enhanced nutrient levels in the soil below cushions is the decomposing dead tissue accumulated within them (Yang et al., 2010). Such a decomposition, however, is presumably rather slow in the very dry and cold climate of the Trans-Himalaya and may not be sufficient to replenish the nutrients utilised by the cushion itself. Cushions, therefore, do not always provide a microsite with better nutrient availability. Moreover, the difference in nutrient concentration might also be caused by the activity of biological soil crusts (Řeháková et al., 2011), which commonly occurred in the open areas of our study region. These are known for their capability of raising nutrient levels (Gold and Bliss, 1995; Guo et al., 2008).

Plant-plant interactions

Since plants compete for the same resources, there would have to be a strong reason for other species to prefer growing inside cushions. We could not prove that cushions ameliorated microsite conditions, a fact which would overshadow the competive pressure. Our results, on the contrary, showed that there were less nutrients in the soil under cushions and a temperature regime comparable to open areas. The only exception was a lower site in Nubra, where the cushion increased the soil temperature below it, however, the plants on the top of the cushions might still have suffered from overheating. Furthermore, *T. caespitosum* cushions are exceptionally hard and compact and this will naturally reduce the opportunity for other plants to establish themselves inside. This brings us to realisation that the observed negative plant-plant interactions might be a result of "cushion quality effect" rather than a climate effect, and that comparison with other cushion species growing in the same environment is needed (Sklenář, 2009). In fact, the probability of being colonised differs among cushion species, which are usually included in various comparisons and generalisations. Typical cushions, e.g. *Azorella* spp., *Laretia acaulis* or *Arenaria polytrichoides*, are often compared to species with relatively loose branches, which allows interacting species to establish more easily. This indicates also that the term ‘‘cushion plant‘‘ is ambiguously understood, at least in this point of view, but this issue is discussed only rarely (Sklenář, 2009).

Our results showed that along the entire elevation range of *T. caespitosum*, the open areas were not only richer in species composition, but not a single species preferred to grow inside cushions. This preference for the habitat outside cushions is in sharp contrast to the results from other studies on cushions (Pyšek and Liška, 1991; Núñez et al., 1999; Badano et al., 2002; Badano and Cavieres, 2006a,b; Zoller and Lenzin, 2006; Badano and Marquet, 2008; Arredondo-Núñez et al., 2009; Quiroz et al, 2009; Anthelme et al., 2012). In addition to the fact that the cushions did not provide a better microhabitat, we ascribe the prevailing negative association between cushions and other plants to the extreme conditions present in our study area.

In the Tso Moriri region, vascular plants grow at one of the highest elevations in the world, occurring up to 6150 m. This is due to the relatively flat terrain of an unglaciated high-elevation plateau bearing well-developed soils. The current record (6400 m, *Saussurea gnaphalodes*) (Miehe, 1991) is actually from a much wetter region of Mt. Everest, where the circumstances of the find indicate that the plant was rather isolated in an exceptionally suitable microhabitat. This is not the case in our study region, where the vegetation is continuous up to 5960–6000 m, consisting of 9 species here, although with a negligible cover (Dvorský et al., 2011). *T. caespitosum* and accompanying species reach their absolute elevational limit here. On the very margins of angiosperm existence, the conditions are so extreme that species inhabiting such places must be stresstolerant and well adapted in order to survive on their own. Moreover, the cushions at high elevations are mostly flat and do not protrude much above the ground, which reduces their possible sheltering effect against desiccating wind and abrasion. Thus, other plants might preferentially establish and grow in protected microsites in the lee of the countless stones instead of the cushions.

The observed trend of species preferring open areas was also consistent in the lower parts of the gradient both in Nubra and Tso Moriri. Although the cold steppes zone here has a more favourable temperature regime, from air relative humidity measurements and the apparent precipitation increase with elevation in combination with uneven evapotranspiration rates, we assume that plants from lower elevations are subject to higher water stress. This assumption was also supported by our pilot study on plant transpiration rates (Doležal, unpublished data). All the species growing here must be well adapted to the arid conditions with water as a limiting factor. Therefore, the species did not need a cushion to survive and preferred the open areas where one can expect lower competition for water.

The negative plant-plant interactions prevailing across the entire elevation range of *T. caespitosum* from 4850 to 5850 m can be explained by the competition for water and nutrients together with negligible or no advantage provided by cushions. The less negative association observed at the upper part of Nubra Valley at 5250 m seems to be linked to less stresfull water and thermal conditions, as indicated by the prevalence of alpine grasslands with high cover.

We suppose, however, that this pattern is a result of cushions being more prone to invasion by other species (those belonging to alpine grassland such as *Poa attenuata*, *Carex pseudofoetida*, *Astragalus strictus*; Table 2) rather than a result of facilitation. *Thylacospermum caespitosum* is a strong competitor in highly stressful conditions, while in mesic conditions (with higher frequency of fast-growing clonal and hence competitive species like grasses and sedges) it is more prone to invasion. The colonisation by other species can be further enhanced by small cracks in cushions caused by abundant yaks which graze on alpine grasslands during the whole summer season.

The observed patterns of plant interactions in this study can be put into the context of ongoing postglacial successional changes in the Himalayas. Our studied cushion species can be called a longlived pioneer; deep roots allow it to colonise infertile glacial substrata early after deposition and then occupy the spot for long time periods due to its slow vegetative spread (unpublished data). The cushions can dominate succession for a very long time, in particular on a stressful site where primary succession towards closed-canopy vegetation, like alpine grassland, is blocked either because the site is dry (low-elevation cold steppes) or very cold (high-elevation subnival zone). If the site is not extremely stressful, *T. caespitosum* is replaced by other species as seen around the old glacial lakes in the upper part of the Nubra locality, where the final stages of postglacial succession are alpine grasslands dominated by graminoids.

Supporting information

Additional supporting information may be found in the online version of this article:

Figure S1 Intensity of interactions between *T. caespitosum* and other species. Calculated using the relative interaction index, competition is represented by negative values and facilitation by positive values. Error bars represent standard errors.

Table S1 Test of species-area relationships. Explained variability (adjusted R^2) from the regressions of log (number of species) (inside and outside cushion) on log (sample area) (i.e. cushion size) in eight elevational sites in Nubra (4850–5250 m) and Tso Moriri (5350–5850 m), with corresponding Type I error estimate (n.s. nonsignificant, $^{a}P<0.1$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$). Shown are also tests of differences in slope and intercept parameter estimates between the two regression lines.

Table S2 PERMANOVA results. Permutational multivariate analysis of variance testing for the differences in species composition between the cushion habitat and open areas.

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CHAPTER VI

Community structure of soil phototrophs along environmental gradients in arid Himalaya

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Photo on previous page: biological soil crusts in subnival zone of E Ladakh, 5900 m a.s.l.

Community structure of soil phototrophs along environmental gradients in arid Himalaya

Abstract

The well-developed biological soil crusts cover up to 40%of the soil surface in the alpine and subnival zones of the Tibetan Plateau, accounting for a vast area of Asia.We investigated the diversity and biomass of the phototrophic part (Cyanobacteria) of the microbial community inhabiting biological soil crusts and uncrusted soils in their surroundings on the elevation gradient of 5200–5900 m a.s.l. The influence of soil physico-chemical properties on phototrophs was studied. The ability of high-altitude phototrophs to fix molecular nitrogen was also determined under laboratory conditions. The biological soil crust phototroph community did not differ from that living in uncrusted soil in terms of the species composition, but the biomass is three-to-five times higher. An increasing trend in the cyanobacterial biomass from the biologicalsoil crusts with elevation was observed, with the genera *Nostoc* spp., *Microcoleus vaginatus* and *Phormidium* spp. contributing to this increase. Based on the laboratory experiments, the highest nitrogenase activity was recorded in the middle elevations, and the rate of nitrogen fixation was not correlated with the cyanobacterial biomass.

Introduction

Soil environments in high-altitude and high-latitude ecosystems provide habitats for numerous microorganisms, despite being subject to extremes of environmental stress, principally freezing and desiccation (e.g. Kaštovská et al., 2005, 2007; Gangwar et al., 2009; Blanco et al., 2012). Phototrophic microorganisms – cyanobacteria and eukaryotic microalgae – are important components of these soil environments, especially in young successional stages (Nemergut et al., 2007; Řeháková et al., 2011). Such microbial communities play a key role in colonizing barren substrates, even above the actual vascular plant altitudinal limit. Here, together with lichens, mosses and microfungi, they persist in a complex structure of biological soil crusts (BSCs) (Paerl et al., 2000; Belnap and Lange, 2001). BSCs carry out key processes in the development of soil (Kubečková et al., 2003; Tirkey and Adhikary, 2005), biogeochemical cycling (Johansen, 1993; Heckman et al., 2006) and plant colonization (Belnap and Harper, 1995). The diversity and abundance of soil cyanobacterial and microalgal communities as first colonizers of high-mountain soils may profoundly affect nutrient availability for pioneer vascular plants by enhancing plant seedling establishment (Belnap and Lange, 2001).

The biodiversity and functions of microbes in the mountain ecosystems have received increased attention, especially with respect to climate change, glacial retreat and vascular plant distributional shift (e.g. Schmidt et al., 2008; Lamb et al., 2011). Climate in most places on the Earth has become increasingly warm over the past two hundred years, with some regions such as high-mountain biomes experiencing an increase of as much as 2–3 K (Alley, 2007). As broad-scale distributions of organisms are shaped by climatic conditions (Walther et al., 2006), changes in climate necessarily result in shifts of range limits. Evidence of such shifts has already been found in a variety of mountain regions and organisms (Parmesen and Yohe, 2003; Root et al., 2003; Walther et al., 2006; Erschbamer et al., 2011).

Most of the available studies, however, have been based on data from European mountains, while the largest and highest mountainous areas are located in Asia. On the Tibetan Plateau, which covers an area almost 14 times as large as the area of the European Alps, extensive areas of subnival and alpine climatic zones host a unique and relatively species-rich flora (Miehe et al., 2011) and well develop BSCs with complex microbial communities (Rˇ eháková et al., 2011). Alpine zones of the Tibetan Plateau serve as grazing land for yaks, wild sheep and other mammals, while many plant species are utilized by local people for medicinal purposes. However, 67% of all glaciers, representing the only reliable water reservoir for local people and their livelihoods, are presently retreating (Rai and Gurung, 2005; Xu et al., 2009). This indicates that considerable environmental change in this high altitude region is underway.

Despite the importance of phototrophs in alpine soils, their biodiversity and functions are still unclear and require further attention, especially in remote mountain regions such as the Tibetan Plateau. The dry mountains in SW Tibet are situated in the rain-shadow north of the Great Himalaya, the so-called arid Trans-Himalaya. These provide unique opportunities for investigating the response of various organisms to the changing environment at one of the highest (c. 6000 m) elevations in the world inhabited by angiosperms (Klimeš and Doležal, 2010; Dvorský et al., 2011). The harsh environment of the region imposes great constraints on plants, which must cope with aridity and salinity at the lower altitudes, while extreme diurnal temperature fluctuations, strong winds and solifluction constrain plants at the higher altitudes. Under such conditions, the productivity is generally very low and the vegetation cover is sparse. These circumstances open the space for the extensive development of phototrophic communities in the BSCs and increase the importance of cyanobacteria and microalgae.

The aim of this study was to assess whether the taxonomic composition and biovolume of phototrophic microbial communities differ between soil crusts and barren soil and whether these differences are constant across an elevational gradient from 5300 to 5900 m a.s.l. covering various habitats, from dry alpine steppes, to wet alpine screes and the subnival zone. We also assessed whether the taxonomic diversity and biovolume of phototrophic microbial communities is linked to soil physico-chemical properties and nitrogenase activity (NA), and vegetation cover. The present investigation represents, to the best of our knowledge, the first study focused on BSCs in the remote region of the Tibetan Plateau.

Methods

Study region

Fieldwork was conducted in August 2010 on the western slope of Chamser Kangri Peak above Tso Moriri Lake (32°59‘N, 78°24’E). This is located in the southwestern extension of the Tibetan Plateau in Ladakh, Jammu and Kashmir States, India (Supplement 2). An arid environment only occasionally affected by monsoon, which seldom cross the main Himalayan range, characterized the whole region. The parent rock consists mainly of siliceous rocks (Tso Moriri gneiss) (Epard and Steck, 2008). The altitudinal zonation of vegetation included steppes at lower elevations (5000–5300 m), alpine screes and meadows (5300–5600), and the subnival zone close to glaciers (5600–6000 m). The cover of vascular plant vegetation ranged from 0 to 50%. The general vegetation description of the study region is provided by Klimeš and Doležal (2010), Klimešová et al. (2010) and Dvorský et al. (2011).

Soil sampling

The sampling was done at four locations along an elevational transect from 5300 to 5900 m a.s.l. so as to cover the physiognomically different major vegetation types of steppes, alpine screes, alpine meadows and the subnival zone. Places devoid of soil and vascular plants such as very steep unstable slopes, glaciers, big boulder moraines and elevations >□6000 m a.s.l. were avoided. At each site on a gradient, the five pairs of composite samples were taken randomly, one from the bare surface layer of soil and the other from soil crust nearby. Soil was taken from an area of 10 cm^2 and 1–3 cm deep, with a sterile spatula. In total, 40 soil samples were collected (4 elevations × 2 soil types × 5 replicates). The soil air-dried on aluminum plates for 10 h immediately after collection, because the field conditions do not allowed other storage of collected material. The BSC and arid land soil are exposed to the drying and freezing commonly in the Himalaya Mts. This method of preservation is recomended for arid land soils and BSC samples by Campbell et al. (2010), because it prevents microbial activity in a naturally occurring manner, without the cell damage that may be associated with freezing and, particularly, thawing cycles. The samples were placed in sterile 100 ml polypropylene bags (Nasco Whirl-Pak®), and transported to the laboratory for analysis.

Physico-chemical characteristics of soil

Subsamples of soil were used for the determination of pH, organic matter content, and texture as described by Kaštovská et al. (2005). For the determination of total nitrogen, NH_4^+, and NO_3^-, methods by Zbíral et al. (1997), Kopáček and Hejzlar (1993), and Wolf (1982) were used. The technique described in Mehlich (1978) was used for the extraction of phosphorus (P) from samples and the concentration of P was measured by using ascorbic acid-molybdate and a SHIMADZU UV-1650PC spectrophotometer. The macro-elements (Ca, Mg, K, and Na) were extracted from the soil according to US EPA method 200.2 ($HCl–HNO_3$) (http://www.epa.gov/epaoswer/hazwaste/test/3050b.pdf) and determined spectrochemically using US EPA method 3050 (Kimborough and Wakakuwa, 1991). Soil organic carbon was determined by wet oxidation with acidified dichromate (Rowell, 1994). Mid-season volumetric water content was measured at each soil sampling point immediately during the collection by a Hydrosense II Soil Moisture Measurement System (Campbell Scientific, Australia).

Algal and cyanobacterial abundance and diversity

The samples for abundance and diversity investigation were prepared as follows. One gram of mixed soil was diluted in 5 ml of distilled water. The slurry was disintegrated at the beginning manually with pestle and consequently with sonificator (Bandelin Sonorex) for 1 min. Twenty microlitre of slurry was put under cover glass cover area 22 × 22 mm. Ten stripes (area of one stripe 11 mm^2) were counted. Samples were observed under microscopy Olympus BX 60, magnification 400×.

The number and biovolume of microalgal and cyanobacterial cells as well as the taxonomic composition of communities were determined using epifluorescence microscopy (Olympus BX 60). Green and blue excitation (MWB filter cube blue excitation 450–480, emission 515+ for eukaryotic algae; MWG filter cube green excitation 510–550, emission 590+ for cyanobacteria) was used (Kaštovská et al., 2005). The term 'eukaryotic' alga is used in this paper for the taxa from classes Chlorophyceae and Tribophyceae. The green coccoid algae included species from the classes Chlorophyceae and Tribophyceae, because it is impossible to distinguish them under the epifluorescence microscope. In our soil samples, it was possible to recognize two morphotypes according different cell dimensions.

Cyanobacteria were classified into three orders according to their morphology: Chroococcales (single-celled organisms), Oscillatorialles (filamentous cyanobacteria without heterocytes and akinetes) and Nostocales (filamentous or colonial cyanobacteria with heterocytes and akinetes). In Oscillatoriales, the taxa *Phormidium* spp., *Leptolyngbya* spp., *Microcoleus vaginatus* and *Microcoleus* sp. were determined according to the width of the filaments, shape of the vegetative cells and presence/absence of mucilaginous sheaths. In Nostocales, two morphotypes were distinguished according to their life form (colonies or filaments) and the shape of the vegetative cells and heterocytes: *Nostoc* sp. and *Nodularia* sp. For the order Chrooccocales, it was possible to recognize taxa *Chroococcus* spp., *Cyanothece* spp. and 'unidentified balls' according to the vegetative cells' shape and dimension, and division of cells. For the dimension of single morphotypes (see Table 1).

Nitrogenase activity

Nitrogenase activity was estimated as acetylene-ethylene reducing activity (Hardy et al., 1973) in all collected soil samples. Stored, dry soils were re-wetted using distilled water; the amount of water necessary to moisten soils to *c.* 24% w/w (23.8 ± 2.3, mean ± SD; moisture is expressed as mass of water per gram of dry soil in percent) was set in a preliminary experiment, details not shown. Thirty gram portions of moist soils were placed in 120 ml serum bottles and pre-incubated under laboratory conditions (22°C, daylight 16 h; day 0). After 24 h, 10 ml of acetylene was added and immediately a 0.5 ml sample of the internal atmosphere was taken with a gas-tight syringe (day 1). Sampling was repeated in the same way after 24 h of incubation (that is on day 2). The bottles were then opened and let to dry for 48 h (days 2–4). Then the determination of NA (days 4–5) and the air-drying (days 5–7) were repeated and finally the NA was determined (days 7–8). The soils were air dried on day 8. Therefore, altogether three subsequent measurements of NA were done using the same bottles with the soils under study. The amount of ethylene in the headspace samples was determined Hewlet Packard 5890 gas chromatograph equipped with a flame ionization detector (FID). A standard mixture of 99.9 p.p.m. ethylene in N_2 was used for calibration purposes. The NA was expressed in nmol C_2H_4 per gram of dry soil per day. For details on ethylene determination and calculations see Šimek et al. (1987).

Microclimatic measurements

At each elevation site, we recorded air temperature and relative air humidity using a HOBO U23 Pro v2 datalogger placed on the soil surface and shielded against direct sunlight. The measurements were recorded every 2 h for the whole year.

Statistical analysis

Differences in cyanobacterial and algal composition between four elevation sites and two soil types (crust and bare soil) were analysed by RDA, which is a constrained ordination method, in the CANOCO 4.5 program (ter Braak and Šmilauer, 1998). RDA was used because environmental variables were in the form of categorical predictors (dummy variables). Standardization by species (dependent variables) was used because the data analysed were of various types and units. The variance partitioning procedure was performed using several RDAs with explanatory variables and covariables to remove their effects and to obtain the net effect of an individual factor. Using this approach, we constructed tests analogous to the testing of particular terms in ANOVA models but for multivariate data; for details, see Lepš and Šmilauer (2003). Four analyses were carried out: (i) Soil type × Elevation (categorial variable, coded as a set of indicator variables) as the sole explanatory variable – the analysis accounts for all, the main effect of soil type, elevation and their interactions. (ii) and (iii) Soil type being an environmental variable, and Elevation a covariable and vice versa – this accounts just for additive (main) effects of the environmental variables. Note that because Soil type and Elevation are orthogonal, the amount of explained variability (i.e. sum of canonical axes) is not affected by the use of a covariable. (iv) The interaction Elevation × Soil is the environmental variable and Elevation and Plot identifiers are covariables. This accounts for the interaction between elevation and site (i.e. for non-additivity of their effects). This corresponds to the interaction in Repeated Measures ANOVA. The significance of these relationships was tested using the Monte Carlo permutation test (999 permutations) constructed for a split-plot design (see Lepš and Šmilauer, 2003, p. 219). The results of multivariate analyses were visualized in the form of a biplot ordination diagram. The relation of soil physicochemical characteristics to cyanobacterial and algal composition was visualized by their passive projection to the RDA ordination plane.

Both RDA with and without standardization by sample norm were used. RDA without standardization reflects both the differences in the species abundances and also the relative proportions of species, while standardized RDA takes into account the proportions of species only. There is an important implication of significant effect in the standardized-bysamples and the non-standardized analyses. The former analysis tests the null hypothesis that there is no effect of the environment on species composition. To reject this hypothesis, it is enough if the absolute values of species abundances differ between treatments, even if the proportion of individual species remains constant. The latter analysis tests the null hypothesis that the relative proportions of species do not differ between sites. The test of the first hypothesis is usually more powerful, but the rejection of the second hypothesis is more ecologically interesting.

The univariate data were further analysed by mixed-effect models. The effect of soil type (crust, bare ground), elevation (5300, 5500, 5700, 5900 m) and their interactions on individual cyanobacterial and algal species/morphotypes was analysed using a linear mixed-effect models or generalized linear mixed-effect models, depending on the nature of a particular response variable (assuming Gaussian, quasi- Poisson, or quasi-binomial distributions). Each pair of samples was considered a ‘main-plot’ and represented a factor with a random effect nested in the elevation site. Soil type and elevation were the fixed effect factors. All the tests were based on the restricted maximum likelihood approach. The statistical significance of the main effects and interactions were assessed by computing Bayesian highest probability (HPD) intervals using Markov chain Monte Carlo simulations (1000 permutations), as this is more appropriate than normal confidence limits for generalized linear mixed models (GLMMs). To control for familywise error rate, the false discovery rate procedure was performed (Benjamini and Hochberg, 1995). Analyses were done using the *lme4* and *language* packages in the R program (R Development Core Team, 2010).

Results

The composition of phototrophic communities

Phototrophic microorganisms were found in all examined samples, with Cyanobacteria being the dominant component of the communities. The phototrophic communities contained 16 morphotypes and the biovolume of a single morphotype ranged from 0 to 490 166 μm^3 mg^{-1} dry weight (DW) (Table 1). Richness and Shannon diversity of soil phototrophic communities significantly decreased with increasing elevation, both in crusts and bare soil (Fig. 1). The total biovolume of soil phototrophs was, however, three-to-five times higher in crusts than in bare soil, and increased significantly with elevation in the soil crusts only (Fig. 1).

The combined effect of elevation and soil type on the composition of soil phototrophic communities explained 40.7% of the total data variation and was highly significant (non-standardized canonical redundancy analysis (RDA): F = 3.006, P = 0.001, Supplement 1). The non-standardized RDA revealed that all morphotypes were much more abundant in soil crusts when compared with bare soils. Variance partitioning revealed that 23.8% was explained solely by soil type differences (F = 13.6, P = 0.001), and 15.2% by elevation (F = 2.89, P = 0.001). Less variation was explained by soil type and elevation in the standardized RDA. A non-significant result of RDA standardized by sample norm shows that, when abundance differences among soil habitats are removed, species proportions remain more or less constant between crust and bare soil in each elevation, while there are significant shifts in species composition along the elevation gradient.

In the RDA ordination diagram from the nonstandardized analysis, the main compositional changes along the first ordination axis are associated with soil type, clearly separating the soil crusts from bare soil (Fig. 2), while the second axis corresponds to the position of the four elevation sites. The RDA diagrams show that cyanobacteria from the orders Oscillatoriales and Nostocales predominated at the higher elevation sites (5700 and 5900 m), while Chroococcales were more abundant in the communities at the lower altitude sites (5300 and 5500 m) (Fig. 2). Hence, Oscillatoriales and Nostocales prevailed in subnival soils, particularly in soil crusts, while Chroococcales were dominant in the soil crusts of alpine steppes and screes at lower elevation (Fig. 2). Eukaryotic algae (Chlorophyceae and Tribophyceae) accounted for a small

Table 1. I. The biovolume of phototrophs in $mm^3 mg^{-1}$ dry soil × 10^3 (means) and II. The nitrogenase activity in nmol C_2H_4 g^{-1} (dry soil) day^{-1}(means) at soil crust and bare soil. Also shown are P-values from GLMM analyses (for Type I error estimate) comparing two soil types, elevation differences and interaction between these predictors. An upward or downward pointing arrow indicates a positive or negative relationship between the dependent variable and altitude or soil (↑ = increase in crust), and ns indicate that the relationship is not significant. ↑in = increase only in soil crust.

	Bare soil	Crust			
	Mean/SDEV	Mean/SDEV	Soil	Elevation	Interaction
I. Phototrophs					
Nostoc sp.	18/8	137/58	↑ 0.015	↑ 0.000	↑in 0.000
Phormidium spp.	44/24	298/144	↑ 0.071	↑ 0.001	↑in 0.015
Microcoleus vaginatus	72/42	490/273	↑ 0.045	↑ 0.002	↑in 0.013
Microcoleus sp.	17/38	110/243	ns	ns	ns
Nodularia sp.	7/7	40/32	↑ 0.000	↓ 0.003	↓in 0.000
Leptolyngbya sp.	4/4	29/17	↑ 0.001	ns	ns
Cyanothece (5 × 10)	$7 \times 10^{-1}/1$	6/8	↑ 0.017	ns	ns
Cyanothece (10 × 15)	0/0	1/2	ns	↓ 0.038	↓in 0.027
Chroococcus 1 (2.5 × 5)	$2 \times 10^{-1}/5 \times 10^{-1}$	1/1	ns	ns	ns
Chroococcus 2 (5 × 5)	$6 \times 10^{-3}/9 \times 10^{-3}$	$4 \times 10^{-2}/5 \times 10^{-2}$	ns	ns	ns
Chroococcus 3 (5 × 7.5)	$5 \times 10^{-3}/0.01$	$3 \times 10^{-2}/5 \times 10^{-2}$	↑ 0.000	ns	ns
Chrococcales (2.5 × 2.5) G1	$1 \times 10^{-1}/8 \times 10^{-2}$	$6 \times 10^{-1}/5 \times 10^{-1}$	↑ 0.001	ns	ns
Chrococcales (5 × 5) G2	1/1	6/6	↑ 0.006	ns	ns
Chrococcales (10 × 10) G3	$2 \times 10^{-1}/4 \times 10^{-1}$	$8 \times 10^{-1}/2$	ns	ns	ns
coccal microalgae (15 × 15) B1	$6 \times 10^{-1}/1$	$3/1 \times 10^{-1}$	ns	ns	ns
Chroococcales	2/2	16/10	↑ 0.000	↓ 0.037	↓in 0.042
Oscillatoriales	137/73	926/414	↑ 0.001	ns	↑in 0.034
Nostocales	26/13	178/77	↑ 0.000	↑ 0.037	↑in 0.028
eukaryotic algae	$6 \times 10^{-1}/1$	3/0	ns	ns	ns
Total biovolume	166/72	1111/392	↑ 0.000	ns	↑in 0.019
II. Nitrogenase activity					
1–2 days	0.11	0.71	↑ 0.080	ns	ns
4–5 days	0.87	2.29	ns	ns	ns
7–8 days	0.04	0.19	↑ 0.038	ns	ns

proportion (0–5.6%) of the total phototrophic biovolume (Table 1) and were more important components of the phototrophic communities of the bare soil at lower elevation (Fig. 2). In the two upper elevation sites, which had the highest biovolume of Oscillatoriales, *Microcoleus vaginatus* and *Phormidium* spp. accounted for most of the biomass (Fig. 3). The biovolume of cyanobacteria

Phormidium and *Microcoleus vaginatus* increased with increasing elevation, but only in soil crusts, while the biovolume decreased with elevation for *Nodularia* and taxa from the order Chroococcales (Fig. 3).

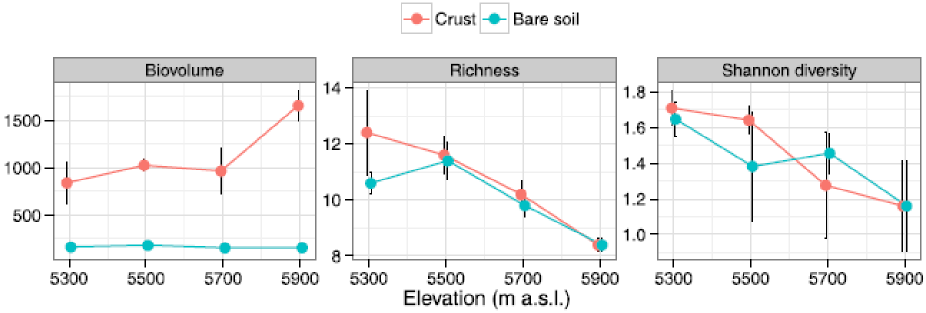

Fig. 1. Comparison of soil phototrophic biovolume (mm^3 × mg dry soil × 10^3), cyanobacterial and algal morphotypes richness and diversity between crusts and bare soil in four elevation sites on the western slope of Chamser Kangri Massif, Tso Moriri Lake region, SW Tibet.

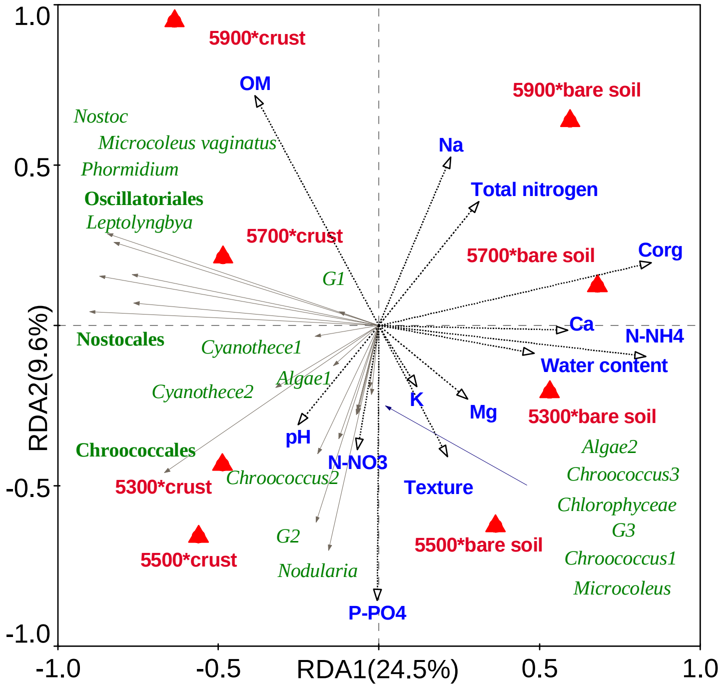

Fig. 2. Redundancy analysis biplots (RDA, not standardized by sample norm) of soil phototrophs (response variables) in relation to soil types (crust, bare soil) and elevation (5300, 5500, 5700, 5900 m) on the western slope of Chamser Kangri, SW Tibet. Response variables are represented by vectors (arrows) and are related to physico-chemical characteristics of soil (dotted arrows) and environmental variables represented by centroids.

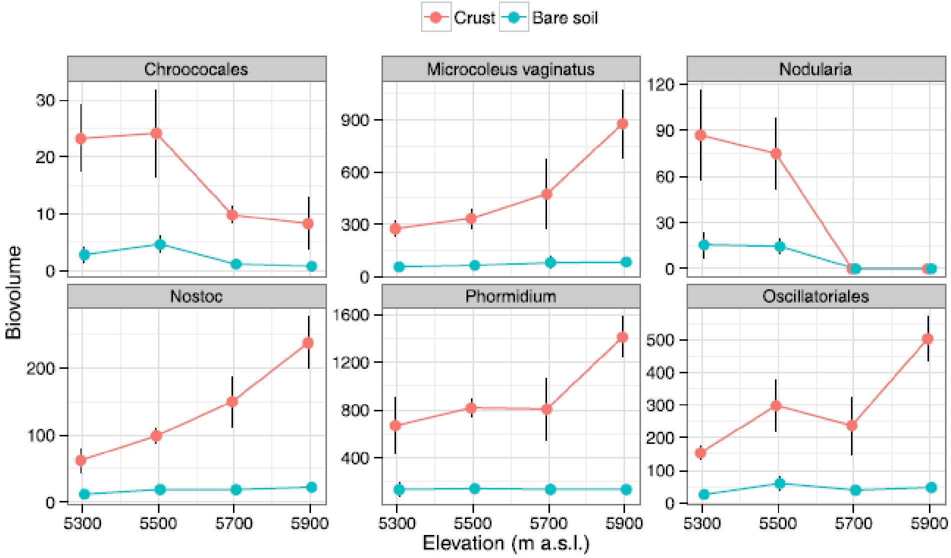

Fig. 3. Comparison of soil cyanobacterial biovolume (mm^3 × mg dry soil × 10^3) between crusts and bare soil in four elevations on the western slope of Chamser Kangri, SW Tibet.

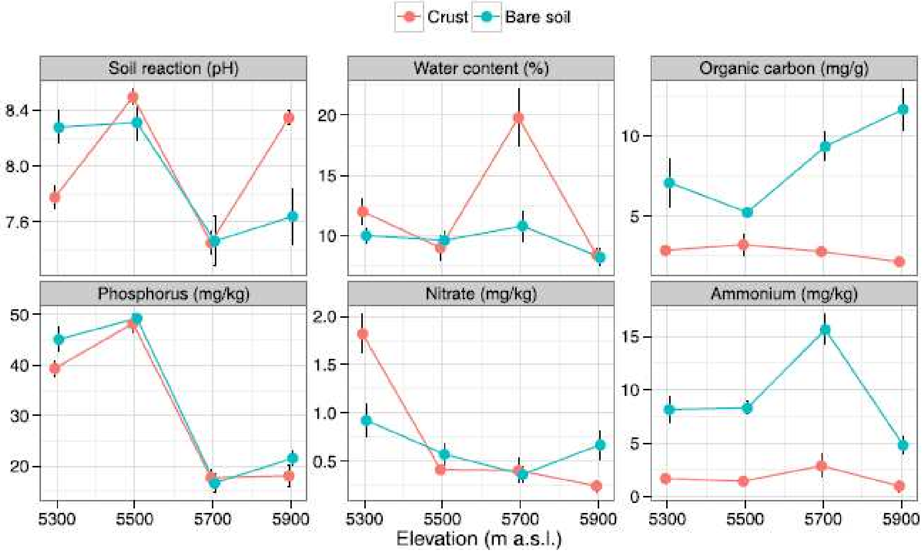

Fig. 4. Comparison of concentration of selected physico-chemical parameters between crusts and bare soil in four elevations on the western slope of Chamser Kangri, SW Tibet.

Soil physico-chemical parameters

The collected soils were variable in their physico-chemical properties (Fig. 4). High pH values indicated alkaline soils in the investigated arid region, which received less than 100 mm of rain per year. This is reflected by low soil water content, ranging from 6% to 20% (expressed as volumetric water content), and high sodium concentration (data not shown). Salt-build-up is common, with saline (NaClaffected) soils found particularly at lower elevations. Soil from lower altitude (alpine steppe and scree) sites had higher nitrate, phosphorous, magnesium and potassium concentrations; with the exception of nitrate, this was particularly true of bare soil. Soil crusts from higher altitude sites had a higher concentration of organic carbon, amonium and calcium in bare soil (Figs 2 and 4). The coarsest soil was found in semi-arid alpine steppes and screes, where the fraction >0.5 mm represented up to 73% of the soil particles; the soil with highest proportion of fine particles was from middle elevations (Fig. 2).

Potential nitrogenase activity in biological soil crusts and in bare soils

This is first time, when the NA was investigated in the nival and subnival zone of Himalaya Mts. N_2-fixing activity (NA) was recorded for most collected soil samples and the ability of Himalaya’s soil to fix nitrogen at least in the laboratory condition was observed. Rates of potential NA (indication of N_2 fixation) are presented in Table 1 and Fig. 5. The results showed that NA depended on elevation, and was, in general, much higher in soil crusts than in bare soil. The highest rates of NA were repeatedly found in soil crusts from an elevation of 5700 m, that is in soils which are the least water limited (Fig. 5).

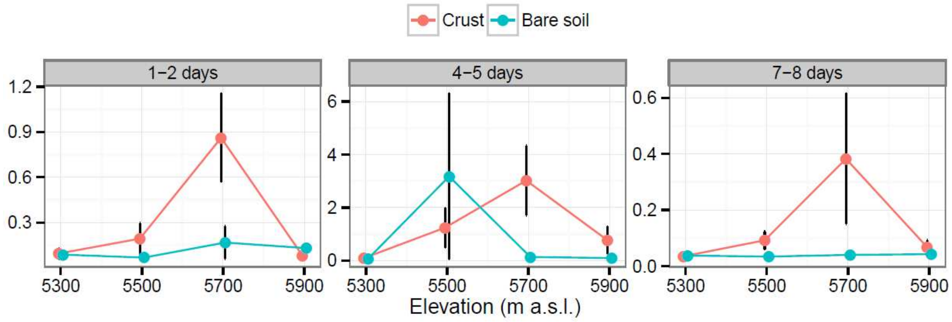

Fig. 5. Comparison of nitrogenase activity [nmol C2H4 g-1 (DW) day-1] between crusts and bare soil in four elevations on the western slope of Chamser Kangri, SW Tibet. Measurements were done repeatedly at the days 1–2, 4–5 and 7–8 of incubation. Heading 1–2 means: start of incubation in day 1 and measurement of NA in day 2. Other two headings had same meaning. The scale in single graphs is different.

The lowest NA values occurred in both the dry and cold ends of the environmental gradient. The range of NA is wide as NA oscillated from 0 to 5 nmol C_2H_4 g^{-1} day^{-1} (Fig. 5). The changes in course of NA in the desiccation experiment were observed only for the altitude 5700 m. NA increased during the first 4–5 days of desiccation, when it reached a rate of 5 nmol C_2H_4 g-1 day-1 and then decreased to 0.3 nmol C_2H_4 g^{-1} day^{-1} towards 7–8 days (Fig. 5). During this time the mass of water per gram of dry soil was initially 5.5%, falling to less than 1%.

Microclimate

The vegetation season in 2010 (defined here as the period with mean daily air temperatures above zero) lasted nearly 5 months (155 days) at the lowest elevation of 5300 m, 3.5 months (107 days) at 5600 m and 3 months (90 days) at 5700 m. At the highest elevation (5900 m) the vegetation season was restricted to less than 2 months (56 days) (Fig. 6).

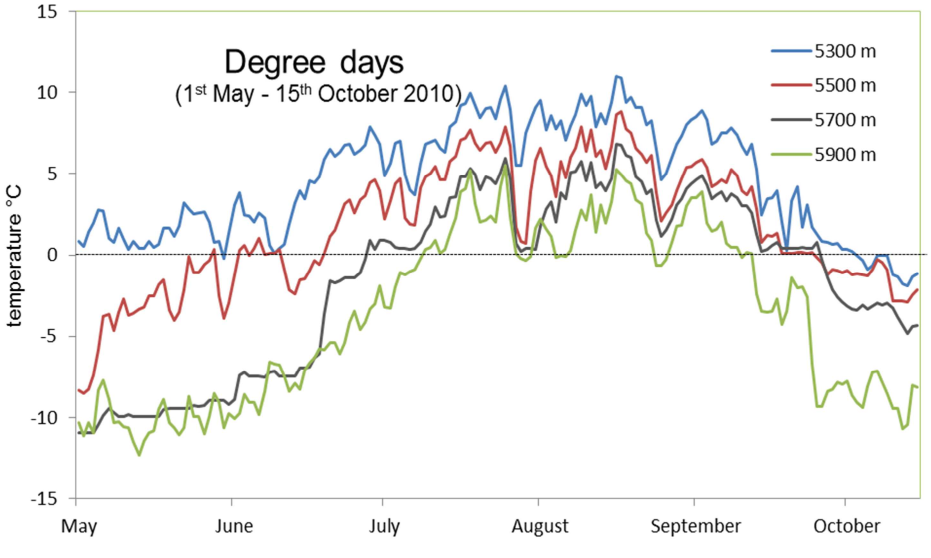

Fig. 6. Mean daily air temperatures measured at four elevations on the western slope of the Chamser Kangri, SW Tibet, Ladakh.

Even during August, when temperatures generally reached their highest value, the temperature regularly dropped below zero at night, and repeatedly to about -5°C at the highest sites. The duration of sub-zero temperatures during a 24 h period was the main difference between the sites. While the temperature never dropped below zero at the lowest elevation, it usually fell below zero for about 2–3 h at the middle elevations; at the highest elevation freezing lasted between 8 and 16 h every day, particularly in the second half of August when many plants still flowered and fruited. Daily temperatures rose to 8–17°C at all four elevations but for a much shorter time per day at higher elevations.

Discussion

Despite an increased interest in the nature of BSCs, there are still many aspects, which need to be studied. One of them is the diversity and role of primary producers (phototrophs) – cyanobacteria and algae – which are the important and dominant components of BSC (Nemergut et al., 2007;Řeháková et al., 2011). The subnival zone of Ladakh is suitable places for development of BSC as almost 40% of soil in this zone is covered by BSC dominated by cyanobacteria. Despite their importance, little attention has thus far been paid to them in the mountain ecosystems of Ladakh.

Phototrophic communities – biomass and diversity

Most of the phototrophs´ biomass was found in the BSC (Fig. 1) at levels three-to-five times higher than in bare soil, even when the measured nutrients had higher concentrations in the bare soil (Figs 2 and 4). Other factors likely contributed to the development of biomass in the biological crusts, or the turnover of nutrients in the crusts may be faster with nutrients being bound only in the microbial loop and not released into the substrata (Schmidt et al., 2007). An interesting finding of our research was a significant increase in cyanobacterial biomass in BSC with increasing altitude, while the biomass in the bare soil did not change significantly through the whole elevational gradient. This increase of cyanobacterial biomass within soil crust with increasing altitude is in sharp contrast to the commonly observed trend of a decreasing biomass of organisms with more severe environmental conditions (Ma et al., 2004; Balser et al., 2010). The observed trend may be caused by several factors. First, there is less competition with vascular plants for resources such as nutrients, water and light. At the highest elevation studied a relatively rich vascular plant community developed, being composed of dwarf rosette species, but with negligible cover of less than 10% (Dvorský et al., 2011). Second, the temperature profile during the vegetation season provides better growing conditions for the microorganisms than for higher plants. The vegetation season lasts about one and half months at the highest elevation (5900 m a.s.l.) and during these days the temperature often drops below freezing for more than 8 h (Fig. 6). The short growing season

and shortage of water are conditions, which microorganisms are resistant to (Zelikova et al., 2012). Phototrophic microorganisms have a much quicker metabolism and a shorter generation time than vascular plants. In addition to this, the low amount of accessible water primarily activates the crust rather than the bare soil (Cable and Huxman, 2004)
where the evaporation is much quicker due to lower retention of water, which is much better in BSC (Aguilar et al., 2009). This also supports the growth of microorganisms in BSC.

The biomass of BSC was mainly created by cyanobacterial taxa, which are important primary producers within soil crusts all around the World. *Microcoleus vaginatus*, *Phormidium* sp. and *Nostoc* sp. have already been commonly reported from arid and semiarid environments (Garcia-Pichel et al., 2001; Yeager et al., 2004; Nagy et al., 2005; Freeman et al., 2009; Abed et al., 2010; Řeháková et al., 2011). Representatives of the order Chrooccocales (*Chroococcus* sp. and *Cyanothece* sp.) account for a minor part of biomass, similarly as in the biotopes from the Antarctic (Komárek et al., 2008). These two taxa are rarely reported from other desert soils in the world. *Chroococcus* creates lesser biomass of phototrophs mainly in the cold types of deserts such as Colorado Plateau or Great Basin desert (Rosentreter and Belnap, 2001) or it is reported from temperate zone from alkaline soils in Ohio (Rosentreter and Belnap, 2001) or salt pasture in Hungary (Büdel, 2001). The species mentioned in the literature above where originally described from water ecosystems or wet dripping walls (*Chroococcus turgidus, C. tenax*). Their occurrence in the desert soil is highly improbable. To solve the proper taxonomic position of Himalaya *Chroococcus* the phylogenetic analyses is needed. Also morphotype *Chroococcus*-like in the deserts of Oman was observed by Abed and colleagues (2010). Cyanobacterium *Cyanothece* was observed in the desert soil previously but also very rarely. We were able to find only two references. First *Cyanothece aeruginosa* from Sand Barrens in Ohio (Rosentreter and Belnap, 2001). *Cyanothece aeruginosa* (Nägel) Komárek 1976 is originally described from clear moorland waters; therefore the correct identification is highly improbable. Second report is cyanobacterium from Solar Lake identified as morphospecies *Cyanothece*-like, called in the article also ‘*Halothece*’ (Abed and Garcia-Pichel, 2001).

Although the species composition of phototrophs is comparable across the investigated altitudinal gradient, their biomass significantly increased (*Microcoleus*, *Nostoc*, *Phormidium*) or decreased (*Cyanothece*, *Nodularia*) with altitude. *Microcoleus* and *Nostoc* are genera, which produce a thick mucilaginous sheath. Besides *Nostoc* has ability to produce scytonemin, which caused brown pigmentations of the sheath (Bowker et al., 2002; Matsui et al., 2012). Both these features protect them against the high UV radiation (Matsui et al., 2012) and desiccation risks (Kubečková et al., 2003) present in the arid environment of the Ladakh Mountains. The genus *Phormidium* produces sheaths only occasionally, but has another protective strategy. *Phormidium* is able to migrate from the surface to the bulk of the soil when the light intensity or moisture level is not suitable for its growth; *Microcoleus* also has this ability (Garcia-Pichel and Castenholz, 2001). The BCS are complex community, where one member of ecosystem plays some role for the other member. The genus *Nostoc*, which grows in the surface of crusts, could protect other, lesspigmented taxa such as *Microcoleus* and *Phormidium* (Bowker et al., 2002). All these adaptations facilitate their survival in soils of subnival zones and enable them to become dominant components of the phototrophic community.

Nitrogenase activity in subnival soils

Crust ecophysiology has not been previously characterized in the region of Ladakh. Because of the welldeveloped cyanobacterial community in the studied soil, where almost 16% of total phototrophic biomass is created by order Nostocales, mainly genus Nostoc, we surveyed their contribution to nitrogen input into the subnival ecosystem. Microbial activity in desert communities is restricted to brief periods when precipitation or dew hydrates microbial cells, thus allowing metabolic activity of the otherwise dormant biota (Garcia-Pichel and Belnap, 1996; Lange, 2001). Temperature is another environmental factor, which influences nitrogen fixation (Gallon et al., 1993) and high UV radiation levels deactivate cyanobacterial nitrogenase (Kumar et al., 2003). To avoid the restriction caused by the unfavourable environmental conditions for NA, we set up controlled experimental conditions (temperature 21°C, full-water saturation of soil, low UV radiation) for NA measurement. All investigated crust samples had detectable NA while almost no activity was detected in bare soils irrespective of elevation (Fig. 5). In spite of the high variability in measured values during the experiment, some significant results were apparent. The NA was independent on the total biomass of cyanobacteria in BSC. The total biomass increased in the BSC with elevation, but NA did not follow this trend. Even if the taxonomic composition of primary producers was comparable across elevation, the intensity of NA differed.

The NA activity was repeatedly highest in crusts from the elevation 5700 m a.s.l., despite the same ‘propitious’ conditions for all measured samples during experiment. The nitrogen-fixing organisms have probably ‘memory’ about *in situ* conditions in natural ecosystems and keep it even in the laboratory. The reason for the highest NA in middle elevations could be that climatic and edaphic factors are more favourable here for NA. BSC are less water-limited than in the lowest (driest) and highest (coldest) elevations studied (Fig. 6). At the same time, the temperature does not drop below freezing so often during the vegetation season as in the 5900 m a.s.l. region (Fig. 6) and the UV irradiance is lower than in the highest elevation.

Crusts from the elevation 5700 m a.s.l. had very similar course of the NA reaction to watering and consequent desiccation as the natural populations of *Nostoc* from Svalbard. Colonies of *Nostoc* were capable of photosynthesis and nitrogen fixation until they lost 40% of their wet weight (Kvíderová et al., 2011). The stability of the NA even with relatively low water content in the cyanobacterial tissue or BSC can be explained by the complex response of microorganisms to desiccation. The major water loss occurs in the mucilaginous envelopes of the colonies or in the intercalary space of the soil during the first days of desiccation but the intracellular water content is not affected (Kvíderová *et al*., 2011).

The majority of organisms are not able tolerate 10% water content treshold in their body, because there is no longer sufficient water to form monolayer around proteins and membrane. Consequently metabolic and enzymatic activities are stopped (Alpert, 2006; Tashyreva and Elster, 2012).

Although the NA determined in laboratory conditions was in general relatively low, not exceeding approximately 6 nmol C_2H_4 g^{-1} day^{-1}, it can be a significant source of N *in situ* in subnival soils. Hrčková et al. (2010) found NA in cyanobacterial species isolated from deglaciated soils in Polar Regions, which were similar to NA in cyanobacteria isolated in temperate and tropical regions. This indicates the potential of cyanobacteria to nitrogen fixate regardless of the environmental conditions they are exposed to.

The conducted investigation in Himalaya Mts. revealed relatively small taxonomical diversity of phototrophs in the studied BSCs and barren soil, nevertheless phototrophs contributed significantly to the biomass of soil microorganisms in subnival and alpine zone. With increasing elevation their biomass and relevance in BSC is growing. Because diazothrofic taxa created up to 16% of total cyanobacterial biomass of BSC, their contributed to the nitrogen cycle in subnival and alpine environment, mostly in the altitude 5700 m a.s.l.

Supporting information

Additional supporting information may be found in the online version of this article:

Supplement 1 Summary of results of redundancy analyses (RDA) for phototrophic soil composition, standardized (S) or not (N) by sample norm.

Supplement 2 Location of four studied elevation sites near Tso Moriri Lake, SW Tibetan Plateau in NW India (top left picture). Studied soil crusts on the western slope of Chamser Kangri Masif at elevation 5700 and 5900 m a.s.l. (top and middle right). Southeastward view of Chamser Kangri (6666 m a.s.l.) with glacier tongue descending to 5700 m.

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CHAPTER VII

Vascular plants at extreme elevations of subnival zone of E Ladakh, NW Himalaya

Dvorský M, Doležal J, Kopecký M, Altman J, Chlumská Z, Řeháková K,
Janatková K (*submitted manuscript*)

Photo on previous page: our base camp in 5600 m a.s.l. in the lower part of a high-altitude plateau in Rupshu region in E Ladakh; the highest occurrence of alpine grasslands.

Vascular plants at extreme elevations of subnival zone of E Ladakh, NW Himalaya

Abstract

We provide a description of subnival zone on the westernost spur of Tibetan Plateau which stretches to Eastern Ladakh, India. The zone ranged from alpine-subnival ecotone (ca. 5600 m a.s.l.) to the highest known occurrence of vascular plants in the region (6150 m). We used 481 floristic relevés collected within a wider region (1 ha, 4600–6060 m, 306 species) to obtain information about regional species distribution and 91 permanent plots (1x1 m, 5560–6030 m, 41 species) to describe vegetation within our study region. We recorded soil, surface and air temperature, soil moisture and air relative humidity along elevational gradient (5600–6150 m). The high-altitude flora consisted of 67 species with prevailing hemicryptophytes. Most represented families were Brassicaceae (16), Asteraceae (11), Poaceae (8), Fabaceae (7), Cyperaceae (6). Species occurring above 6000 m belonged to genera *Aphragmus*, *Draba* (2), *Ladakiella*, *Poa*, *Saussurea* (2), *Stellaria* and *Waldheimia*. A half of the permanent plots contained less than 5 species and with total plant cover below 20 %. Plots above 5900 m never exceeded 5 % in cover and contained 2 species on average. Strong diurnal temperature fluctuations ruled the microclimate. Absolute recorded minimum was -38.3 °C, absolute maximum 31.9 °C, maximum daily range 42.4 °C. Growing season lasted between 88 and 153 days (coldest and warmest site). Mean T of the soil during growing season ranged between 2.9 and 5.9 °C. Sum of heat (above 0 °C) ranged between 245 and 916 degree days. Snowfall and frosts were common throughout growing season.

Key words: Transhimalaya, Changthang, Little Tibet, alpine microclimate, high-altitude flora

Plant scientific names follow Klimeš and Dickoré (2006).

Introduction

Subnival vegetation zone in its typical form (e.g. as known from the Alps) is situated above the closed alpine grasslands and consists of scattered vegetation patches and isolated plants up to the highest growing individuals (Körner 2003). In arid mountains its lower boundary may be obscured and not as clear as in wet regions because of the absence of closed grasslands (Hartmann 1995). Conditions at extreme elavations are stressfull and plants must be adapted to low temperature (T), short vegetation season, high irradiation, strong dessicating wind and abrasion, solifluction and frost heave, snowfall during vegetation season, nutrient-poor substrates and low partial CO_2 pressure (at 6000 m ca. half compared to the sea level). Globally, suitable conditions for a proper vegetative growth and reproduction of angiosperms, i.e snow-free periods and T above 5 °C during vegetation season, can be found up to about 6000 m (Körner 2003).

In Himalaya, plants occur even above 6000 m, with the absolute known vertical limit at 6400 m reached by the species *Saussurea gnaphalodes* (Asteraceae) found in 1938 by E Shipton in the region of Mt. Everest (Miehe 1991, General Herbarium, British Museum, London). However, records of plant species above 6000 m are usually obtained accidentally by climbing expeditions and knowledge about angiosperm life at the highest elevations is thus very limited. Scientific studies on the vegetation and climate from elevations above 5000 m are extremely rare. Modern geobotanical survey of E Ladakh was carried out by Hartmann (1987, 1990, 1999). He was, however, focused on zonal vegetation of lower elevations and never worked above 5300 m, omitting the unique vegetation of high-alpine and subnival zones. Due to the low state of research, Klimeš started systematic floristic explorations of E Ladakh and significantly extended the checklist of species known from this region (Klimeš and Dickoré 2006). Recently, he also discovered plant species new to science here (Al-Shehbaz 2002; Kirschner et al. 2006; Klimeš and German 2008). In the neighbouring region of Tibet, Baniya et al. (2012) explored patterns of plant species richness and composition along an exceptional elevational gradient (4985–5685 m) on Buddha Mountain near Lhasa, however, they did not mention where the upper elevational limit of angiosperms in the region was. Other studies on vascular plant richness in high-elevations of Tibet and Nepal were based on secondary data extrapolated from published floras (Grytnes and Vetaas 2002; Baniya 2010).

To the best of our knowledge there is no study from Himalaya based on original data which would systematically cover the subnival zone from its lower end up to the highest growing vascular plants. The goal of our study is therefore to fill this gap. Our study region is well-suited for such a study because it is one

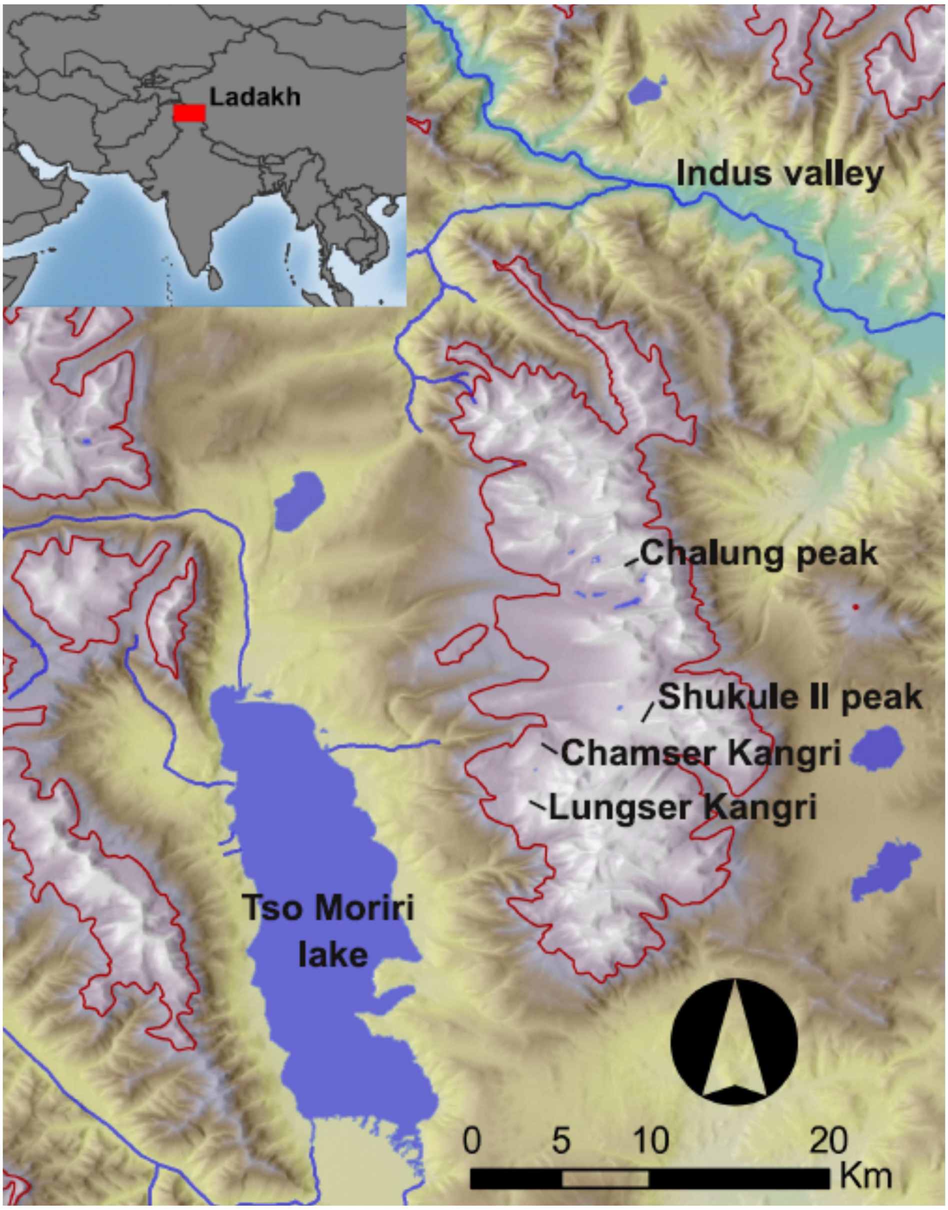

Fig. 1. The study region comprises the high-altitude plateau east of Tso Moriri lake. Blue colour indicates water resources (lakes, streams, rivers), the contour line at 5600 m is depicted by red line, names of important peaks are shown

of the highest places on earth which support angiosperm life. Thanks to the relatively flat terrain (high altitude plateau) and arid climate (permanent snowfields and glaciers begin at higher elevations than in humid regions), there are habitats suitable for vascular plants fairly above 6000 m. The whole region, due its remoteness and extreme conditions, remains unaffected by plant invasions. Moreover, altitudes above ca. 5600 m are practically free of any direct human impact, even if occasional herd transhumance or grazing may occur.

We present a description of plant assemblages, distribution of typical species and their growth characteristics in the context of the abiotic environment. We also discuss constraints and limitations which stand behind the current elevational limit of vascular plants in the region.

Methods

Study region

The study region is a part of the Indian Transhimalaya (Jammu and Kashmir State) and it is located within the region of Changthang in Eastern Ladakh, also called Little Tibet (32°59´ N, 78°25´ E). It lies within a mountain system east of Tso Moriri lake and geomorphologically belongs to the Tibetan Plateau, forming its westernmost spur (Fig. 1). It consists of a high altitude plateau (ca. 40 km long from north to south and 20 km wide from west to east at its widest place), with a north-to-south oriented chain of peaks exceeding 6000 m, three of them higher than 6500 m (Chalung 6520 m, Chamser Kagri 6622 m, Lungser Kangri 6666 m). The plateau is surrounded by plains with several brackish lakes and descends to the bottom of Indus river valley in the east (4600 m).

The whole region is generally arid as the eastern Ladakh is rarely affected by monsoonal precipitation, which is blocked by the main Himalayan range. Unfortunately, there are no precipitation measurements for the region. We can, however, infer from the data recorded in nearest meteorological stations (Leh: 115 mm/yr, 3514 m, ca. 50 km NW of the study region, Gar: 54 mm/yr, 4232 m, ca. 160 km SE of the study region) that the annual precipitation amount is less than 100 mm. Most precipitation falls in summer, above ca. 5000 m mostly in the form of snow. Winter precipitation is erratic and the snow layer is usually thin (Harris 2006). As the precipitation generally increases and temperature/evaporation decreases with elevation, we suppose that the vegetation above ca. 5500 m is not limited by water availability as it might seem from the low precipitation amount. Measurements in Tso Moriri region in 2003 showed that the soil T at the upper limit of vascular plants at 6030 m remained above zero only between mid-July and the beginning of September (Klimeš and Doležal 2010). The substrates vary from siliceous rocks (Precambrian granites, Tso Moriri gneiss) to calcareous or saline sediments at the plains around endorheic lakes. Soils are characteristic by the coarse-grain structure with high percentage of bigger gravel, low water content and organic matter, higher pH

(7.5–9) and relatively high concentration of macronutrients (Řeháková et al. 2011). Biological soil crusts are a typical feature of local soil life (Janatková et al. 2013).

In E Ladakh with its arid climate the delimitation of vegetation zones is indistinct (Hartmann 2009). Forests, the upper end of which usually indicate the beginning of alpine zone, are not present here and large spatial heterogeneity of water availability causes great discontinuities. The alpine-subnival ecotone, as usually defined (e.g. Körner 2003), is not obvious here because closed alpine grasslands are present only locally along streams and do not form a continuous belt. Vegetation is open and scarce along the whole elevational gradient, so that the transition between closed grasslands and patchy subnival vegetation does not exist (Hartmann 2009). The alpine-subnival ecotone neither coincide with the climatological concept of snow-line like in the Alps (e.g. Gottfried et al. 2011) and is situated markedly lower than the traces of snow remaining during average summer. The upper boundary of subnival zone (upper limit of angiosperm distribution in our sense) is set by a combination of various factors rather than being demarcated by permanent snow. The plains around lakes are covered with semideserts, gradually changing into cold steppes with increasing elevation. At dry habitats (most of the region) these cold steppes go up to about 5600 m where typical steppe species like *Artemisia minor*, *Oxytropis tatarica*, *Stipa subsessiliflora* or *S. caucasica* disappear (Dvorský et al. 2011). Therefore, we consider this elevation as the beginning of (upper) alpine zone. The situation at wet habitats (minor area) is somewhat similar. The centre of alpine vegetation zone represented by closed alpine grasslands lies along glacier streams between 5200 and 5600 m and can be best characterized by the distribution of species like *Kobresia pygmaea* and *K. schoenoides.*

Therefore, in search of typical subnival vegetation, we focused on elevations above 5600 m. Above this elevation, the water availability very likely ceases to be the major factor differentiating the species assemblages and the low T becomes the limiting factor for plant survival. In other words, a search for water turns into a search for warm. Vascular plants occur continually from banks of Tso Moriri lake and valley bottoms (4600 m) up to the highest recorded occurrence at 6150 m (SW slope of Shukule II peak, Fig. 2). However, other peaks remain poorly explored and higher records can be expected.

Vegetation sampling

Our knowledge of the floristic composition and the altitudinal distribution of species is based on the following:

a) 481 floristic relevés (100x100m) recorded in a wider context of the study region (the mountain plateau with its immediate surroudings down to the bottoms of the valleys) during years 1999–2002 by late Leoš Klimeš and covering elevational range 4600–6060 m (Fig. 3); 330 species were identified in total, after excluding non-native weeds, cultivated crops and dubious species 306 species were used for further analyses.

b) 91 permanent plots (1x1m) with complete species composition and estimated percentage cover; recorded systematically on the eastern slopes of Chamser Kangri peak at the upper part of the Lapgo valley (Fig. 2) in 2009 and covering range 5560–6030 m; 13 plots were sampled above 5900 m and because of extremely low vegetation cover, these upper plots were selected non-randomly in order to contain at least some plants; 41 species were recorded

c) exploration of several high peaks in search of the highest-growing vascular plants

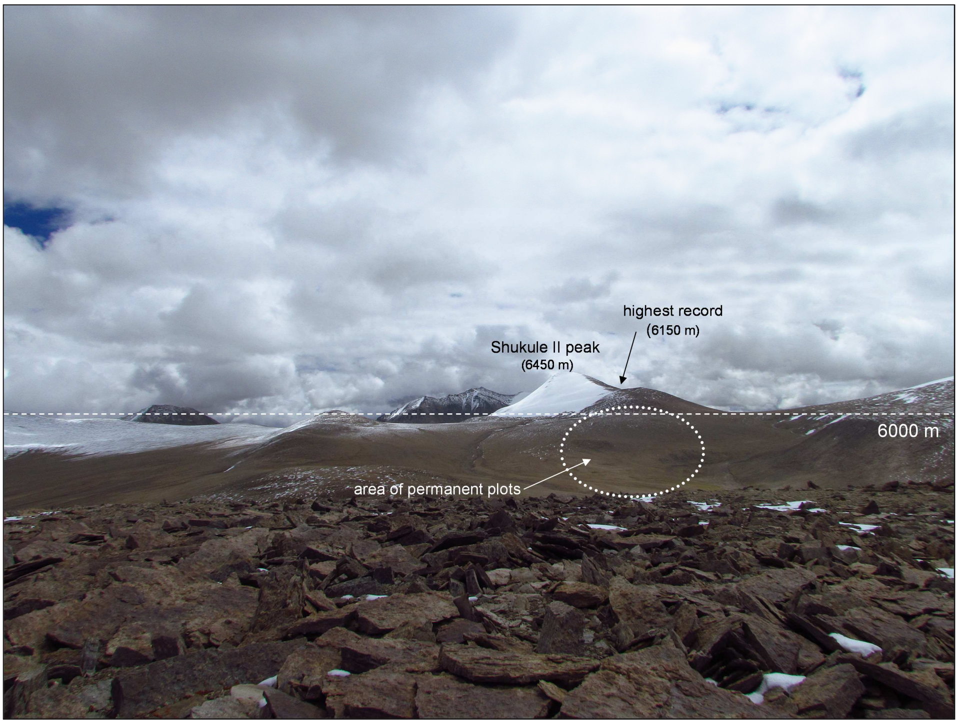

Fig. 2. Study region. Western slope of Shukule peak with adjacent part of the plateau. The scattered line represents the contour line at 6000 m a.s.l. The black arrow points at the highest known site with vascular plants in the region (6150 m, southwestern slope). The white dotted oval indicates the area with permanent plots and dataloggers

We used the data from permanent plots to calculate floristically based nivality index (Gottfried et al. 2011) to help us recognize the alpine-subnival ecotone. The accumulated sum of heat during growing season was counted as sum of T above 0 °C (measured hourly) and rounded to whole degree days.

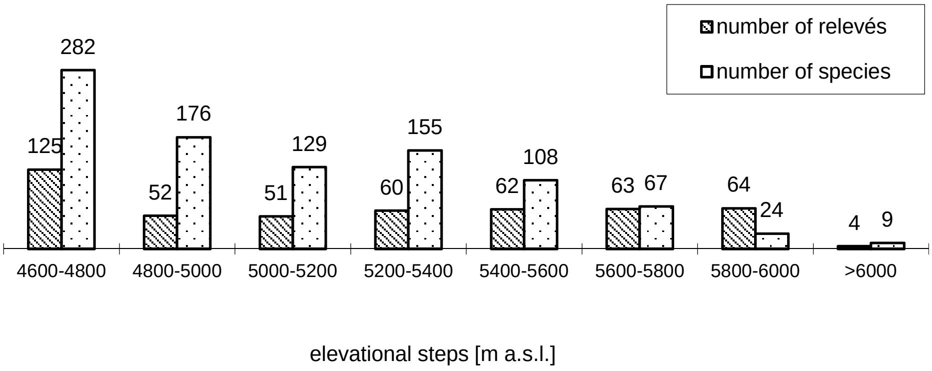

Fig. 3. Number of sampled relevés (1 ha) and total species number within respective elevational steps in Rupshu region, E Ladakh. Altogether, 306 species were found in 481 samples in a range 4600-6060 m (data from L. Klimeš)

Climatic measurements

We used TMS loggers (http://www.tomst.com) to record T of soil (10 cm depth), surface and air (10 cm above). The loggers were recording hourly values from August 2011 to August 2012 and were placed at 5600 m, 5820 m, 5895 m and 6150 m (the site of the highest plant occurence). The sensors were equipped with a white shade so that the T records were somewhat lower to what plants at the surface really experienced. Other data were collected from August 2010 to August 2011 at 5895 m (four TMS loggers on slopes of a flat summit). Furthermore, we measured air T and relative humidity (RH) by a HOBO U23 Pro v2 logger (www.onsetcomp.com) at 5900 m from August 2008 to June 2013 (3 cm above ground, shaded). For comparison, we recorded T by TMS loggers at five additional sites at lower elevations (scree and semidesert at 4700 m, steppe at 5000 m and grassland and scree at 5300 m).

In 2011 and 2012 we placed an automatic PlantCam camera (www.wingscapes.com) on a slope facing the plateau with permanent plots and climatic loggers. The camera was set to take a photo every day at 10 a.m. in order to bring a picture of the snowfall situation. Due to technical failures, we obtained photos only for period from August 2011 to January 2012 and from

August 2012 to November 2012. Nevertheless, the available photos enabled us to correlate RH measurements with snowfall events. RH values usually fluctuate in a course inverse to T. At night, when T is low, RH is highest, with values often exceeding 90 %. As T rises during the day, RH decreases, often below 40 % at midday. After a snowfall, RH values remain relatively constant (Online resource 1). Therefore, we calculated mean daily RH and RH daily variance and used several thresholds to anticipate the real snow situation according to the photos. Finally, we selected RH daily mean above 80 % as the best proxy for the snowfall. This allowed us to extrapolate the snow situation for the whole season.

Results

Species richness and cover

In total, 306 autochtonous species were found within a wider context of our study region (4600–6060 m), 67 of them above 5600 m (Online resource 2). The permanent plots captured 41 of these species, i.e. 61 % of the local high-altitude flora.

Species richness and vegetation cover in plots decreased with elevation (Fig. 4 and 5a). Richness and cover were correlated only little ($R^2 = 0.13$). The highest recorded number of species per $1m^2$ plot was 15 (lower subnival belt, 5607m), although nearly one half of the plots contained less than 5 species. Twenty four species were found above 5800 m (Table 1), 20 species above 5900 m, 9 species above 6000 m (Fig. 6) and 5 species at 6150 m.

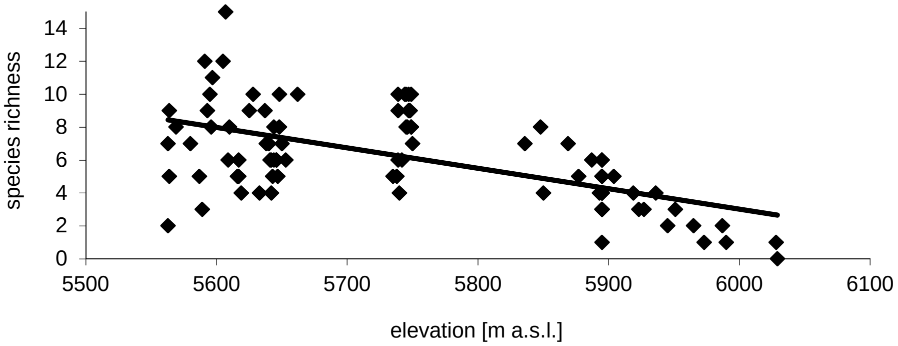

Fig. 4. Species richness in permanent plots in relation to elevation in E Ladakh (n = 91, $R^2 = 0.34$)

Vegetation cover within permanent plots ranged 0.01–100 %, however, one half of the plots had its cover less than 20 %, one third less than 10 % and the cover of plots above 5900 m never exceeded 5 % (Fig. 5a,b). The plots with cover above 50 % (n = 17) were all recorded below 5740 m and in most cases contained clonal species *Carex sagaensis* and *Kobresia pygmaea*. This vegetation occurred in wet habitats along streams and formed alpine grasslands, characteristic for lower elevations.

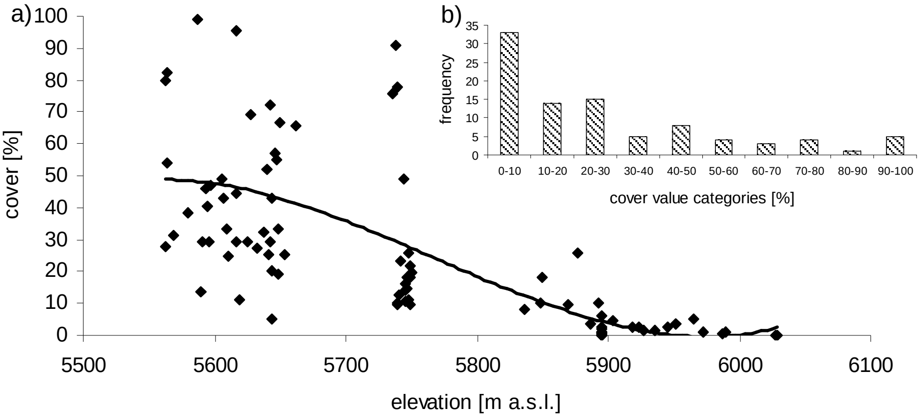

Fig. 5. Vegetation cover in permanent plots in E Ladakh (n = 91, R^2 = 0.45). a) vegetation cover in relation to elevation, b) frequency of respective cover value categories

Floristic composition

For practical reasons of delimiting the characteristic subnival flora we set a boudary at 5800 m. This conventional boundary was surpassed by 24 species (Table 1). Brassicaceae was the most represented family (10 species), followed by Asteraceae (4), Caryophyllaceae (4), Saxifragaceae (3), Boraginaceae, Poaceae and Rosaceae (1 species each). Great differences were found in the vertical range of these species. Half of them had their range greater than 1200 m, including species reaching elevations above 6000 m (*Draba altaica*, *D. oreades*, *Poa attenuata*[1], *Saussurea glacialis*, *Stellaria decumbens* and *Waldheimia tridactylites*). On the other hand, species like *Draba alshehbazii*, *D. glomerata, D. himachalensis* or *Saxifraga stella-aurea* hardly exceeded a range

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[1] note that *Poa attenuata* Trin. is often considered a complicated polymorphic complex with unresolved taxonomy so its wide ecological amplitude in our study region may refer to the existence of vicariant microspecies

Table 1. List of species found above 5800 m a.s.l. Elevational distribution (max - maximum, min - minimum, centre), description of growth forms and habitat demands are shown. Ch-chamaephyte, G-geophyte, H-hemicryptophyte, T-therophyte. Classification into growth forms is according to Klimešová et al. (2011) where details concerning adaptations of respective growth forms and their distribution in Eastern Ladakh are provided (see also Chapter III of this thesis).

	family	**min**	**max**	**range**	**centre of distribution**	**growth form**	**description**	**ecology**
Aphragmus oxycarpus	Brassicaceae	4540	6000	1460	alpine	A. euchroma	H with caudex, forming small dense cushions	gravel river bed sediments
Arenaria bryophylla	Caryophyllaceae	4650	5920	1270	alpine	T. caespitosum	Ch with caudex, forming dense cushions	various types of dry and mesic habitats
Desideria pumila	Brassicaceae	5380	5990	610	subnival	S. gnaphalodes	H with a pleiocorm of long below-ground branches	rock crevices, stabilized slopes
Draba alshehbazii	Brassicaceae	5665	5995	330	subnival	T. caespitosum	H/Ch with caudex, forming cushions	rock crevices, stabilized slopes
Draba altaica	Brassicaceae	4540	6150	1610	upper alpine/subnival	T. caespitosum	H/Ch with caudex, forming cushions	rock crevices,stabilized slopes
Draba glomerata	Brassicaceae	5530	5850	320	upper alpine/subnival	T. caespitosum	H/Ch with caudex, forming cushions	rock crevices, stabilized slopes
Draba himachalensis	Brassicaceae	5850	5960	110	subnival	T. caespitosum	H/Ch with caudex, forming cushions	rock crevices, stabilized slopes
Draba oreades	Brassicaceae	4800	6010	1210	subnival	T. caespitosum	H/Ch with caudex, forming cushions	around springs and snow beds
Eritrichium hemisphaericum	Boraginaceae	5350	5990	640	upper alpine/subnival	M. tibetica	H with caudex, forming small dense cushions	stabilized wind-exposed slopes
Ladakiella klimesii	Brassicaceae	5350	6150	800	subnival	M. tibetica	non-clonal H with perennial main root, forming a cushion	rock crevices, stabilized slopes
Pegaeophyton scapiflorum	Brassicaceae	5270	5940	670	upper alpine/subnival	P. nudicaulis	G with a rosette, main root with adventitious buds sprouts to vertical stems	permanenly wet solifluction soils
Poa attenuata	Poaceae	4540	6000	1460	alpine	F. kashmiriana	caespitose H, forming dense tussocks	stony steppes, dry river beds, screes, mesic turf
Potentilla pamirica	Rosaceae	4540	5890	1350	alpine	A. euchroma	H with short epigeotropic rhizomes, forming dense groups of shoots	mesic stony slopes, gravel sediments
Saussurea glacialis	Asteraceae	4596	6150	1554	upper alpine	S. gnaphalodes	H/G with caudex, develops long unrooted below-ground stems, forming stands with low density of rosettes	unstable slopes and screes
Saussurea gnaphalodes	Asteraceae	5300	5970	670	subnival	S. gnaphalodes	H/G with caudex, long unrooted below-ground stems form low-density stands	screes, unstable gravel slopes, solifluction soils
Saussurea hypsipeta	Asteraceae	5250	6150	900	subnival	A. euchroma	non-clonal H with perennial main root	unstable slopes and screes
Saxifraga cernua	Saxifragaceae	4650	5890	1240	alpine	V. kunawurensis	H with a short epigeotropic rhizome, pseudovivipary	solifluction soils, brook banks
Saxifraga nanella	Saxifragaceae	5300	5995	695	subnival	V. kunawurensis	rosette H/Ch with short epigeotropic rhizomes, forming cushions	snow beds and wet rock crevices
Saxifraga stella-aurea	Saxifragaceae	5670	5990	320	subnival	V. kunawurensis	rosette H/Ch with short epigeotropic rhizomes, forming cushions	snow beds and wet rock crevices
Smelowskia tibetica	Brassicaceae	4830	5830	1000	alpine	M. tibetica	T, usually prostrate with numerous branches initiated at plant base	snow fields, unstable soils disturbed by needle ice
Stellaria decumbens	Caryophyllaceae	4560	6060	1500	subnival	S. gnaphalodes	H with extensive lateral spread of shoots, forming a cushion	stony areas and slopes
Stellaria depressa	Caryophyllaceae	4540	5950	1410	alpine	S. gnaphalodes	H with long rooting stems forming low-density stands to dense cushions	open-canopy stands, stabilized slopes
Thylacospermum caespitosum	Caryophyllaceae	4550	5960	1410	upper alpine	T. caespitosum	Ch with caudex, forming dense and large cushions, withered leaves persist on plant stems, filling the room between them	mesic to wet stabilized slopes
Waldheimia tridactylites	Asteraceae	4820	6150	1330	upper alpine/subnival	S. gnaphalodes	H with long hypogeotropic rhizomes and short epigeotropic rhizomes initiated at soil surface	gravel screes, mesic slopes and rock crevices

of 300 vertical metres (Table 1). Some species had the centre of distribution below 5800 m and were more typical for the alpine zone, e.g. *Arenaria bryophylla*, *Potentilla pamirica, Saxifraga cernua* or *Stellaria depressa* (Fig. 7). Other species had their optimum above 5800 m and can be considered subnival specialists, e.g. *Desideria pumila*, *Ladakiella klimesii, Saussurea hypsipeta* and most of *Draba* species (Fig. 7).

Fig. 6. Species found above 6000 m a.s.l. (photo Miroslav Dvorský and Leoš Klimeš). 1 – *Actinocarya acaulis*, 2 – *Draba altaica*, 3 – *D. oreades*, 4 – *Ladakiella klimesii*, 5 – *Poa attenuata*, 6 – *Saussurea glacialis*, 7 – *S. hypsipeta*, 8 – *Stellaria decumbens*, 9 – *Waldheimia tridactylites*

Hemicryptophytes in their typical form prevailed (11 species, Table 1). A group of species on the transition between hemicryptophytes and chamaephytes was also well-represented (n = 7, *Draba* species and *Saxifraga nanella* group); these plants formed tiny cushions of densely to losely grouped branches or rosettes, with leaves persisting or withering. Typical chamaephytes were represented by cushion plants of greater size and persisting leaves (*Arenaria bryophylla, Thylacospermum caespitosum*). Two species were on the transition from hemicryptophytes to geophytes, having a caudex and long below-ground unrooted stems forming low-density stands of rosettes (*Saussurea glacialis, S.*

gnaphalodes). One geophyte was found (*Pegaeophyton scapiflorum*), resprouting from adventitious buds on the main root, typical for permanently wet soils. A therophyte was also represented (*Hedinia tibetica*, in some sources however considered a perennial plant). One more therophyte of high elevations, although not found above 5650 m, was *Actinocarya acaulis*.

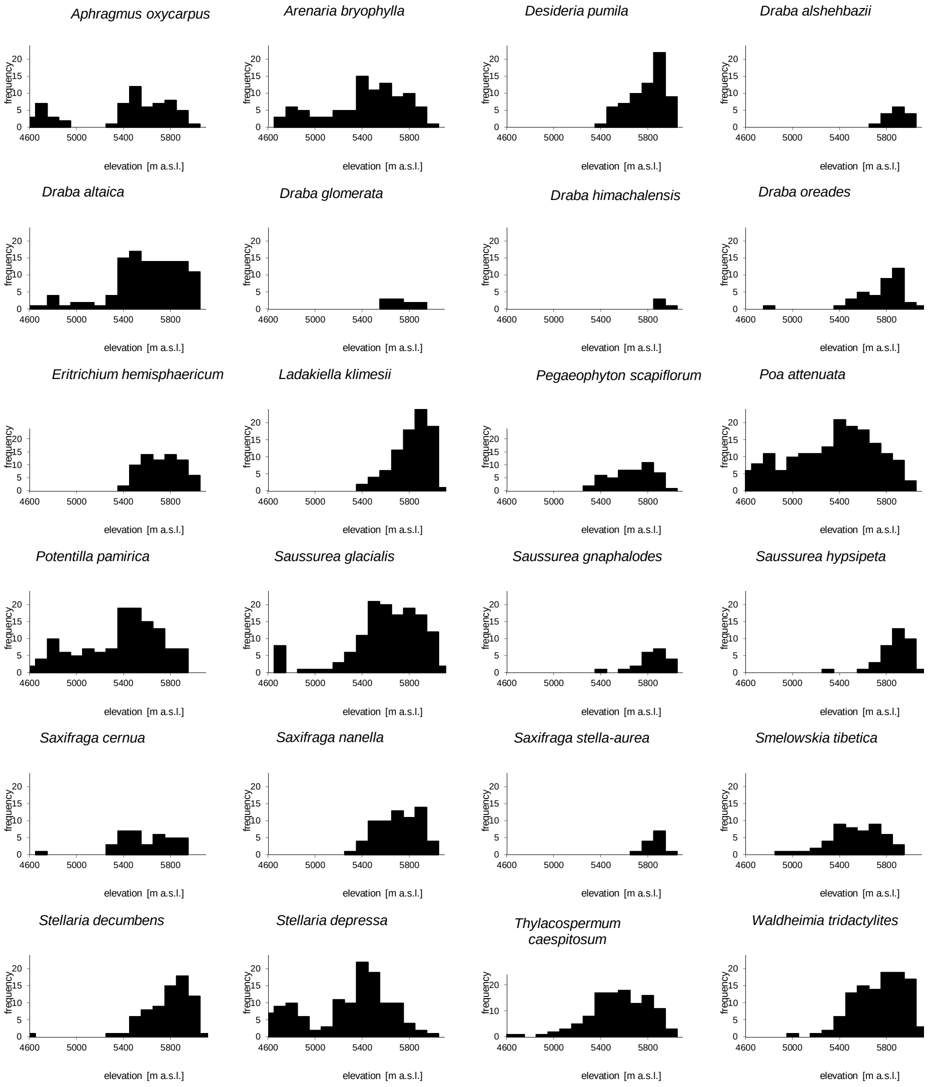

Fig. 7. Vertical distribution of species in Rupshu region, Eastern Ladakh. Respective bars represent frequency of occurence within elevational steps of 100 m. Only species found above 5800 m a.s.l. are shown. Data are based on 481 floristic relevés (1 ha, 4600–6060 m)

Ecological demands of these species seemed to be similar; this was because of the general uniformity of substrates and microhabitats at elevations above 5800 m. Lower, where many of these species had the centre of distribution and where there was a greater habitat variability, their ecological demands could be better observed (Table 1). Above 5800 m, however, DCA analysis showed no substantial differences in the species composition of permanent plots. The only places with slightly different species composition were those with a surplus of meltwater characterized by high frequency of *Pegaeophyton scapiflorum*.

Climate

General microclimate characteristics for sites at 5600 m, 5820 m, 5895 m and 6150 m are summarized in Table 2. For comparison, data from lower elevations of the same slope of the plateau are provided (Online resource 3). Since the subnival plants were never taller than a few centimetres, air T was rather irrelevant for them and we mentioned it just to illustrate the sharp gradients in this environment and to stress the importance of a sheltered microsite.

Strong fluctuations of T were the main feature of the microclimate (Fig. 8). Mean daily range of surface T was 23.9 °C at 5820 m and 15.5 °C at 6150 m. At the lowest measuring site, situated in a boulder field and sheltered by stones, it was only 3.5 °C. Combination of strong solar radiation and neglectable vegetation cover meant that the substrates heated up easily. Due to thin snow cover, above-zero surface T could occur on clear days in February or December while the night temperatures fell below -20 °C on the same days. Strong irradiation and wind, on the other hand, caused rapid cooling and frosts were common throughout the growing season.

The highest site with angiosperms was the coldest in many aspects. The microclimate of the remaining three sites, however, reflected rather their specific microtopography and the second highest site had warmer microclimate than sites below it (Fig. 9). Mean annual T of the soil ranged between -5.4 (5895 m) and -8.7 °C (6150 m). Also T extremes reflected the microsite features (exposure, vegetation cover, shading by stones and the thickness of snow cover) rather than the elevation itself. Absolute minimum T was -38.3 °C (5820 m, surface), absolute maximum 31.9 °C (5895 m, surface), maximum daily range 42,4 °C (6150 m, air, 15.6.2012, min -14.1, max 28.3 °C). January was the coldest month, July the warmest at all sites.

The three sites at the same elevation (5895 m) but different exposure of the same peak (5900 m) showed largely variable microclimate in the measuring

elevation [m a.s.l.]	5600			5820			5895			6150		
exposure	north			west			east			southwest		
temperature [°C]	soil	surface	air	soil	surface	air	soil	surface	air	soil	surface	air
Annual mean	-5.5	-5.9	-6.3	-6.7	-8.0	-8.6	-5.4	-6.7	-7.8	-8.7	-9.1	-10.0
Absolute maximum	11.3	20.3	24.1	23.8	28.3	22.1	24.2	31.9	26.3	16.4	28.4	28.3
Absolute minimum	-18.4	-19.4	-27.8	-29.0	-38.3	-36.8	-29.0	-35.6	-37.0	-24.6	-29.8	-36.0
Maximum daily range	4.9	14.8	23.6	22.8	36.7	32.7	21.1	35.3	35.4	15.1	31.4	42.4
Mean daily range	1.2	3.5	7.8	11.3	23.9	19.0	11.1	21.8	20.8	6.0	15.5	22.4
Mean annual maximum	-4.9	-3.6	-1.3	-0.2	6.1	2.1	0.4	6.1	3.9	-5.5	0.3	3.0
Mean annual minimum	-6.1	-7.1	-9.1	-11.5	-17.8	-16.9	-10.6	-15.6	-16.8	-11.4	-15.2	-19.4
Mean of July	6.2	6.3	7.6	7.2	5.9	5.1	10.4	8.7	6.7	4.2	5.0	3.5
Mean of January	-16.8	-17.4	-19.6	-21.7	-22.8	-22.9	-20.9	-22.0	-22.7	-20.9	-22.6	-23.8
growing season [days]	107 / 107			122 / 110			153 / 147			88 / 88		
seasonal mean [°C]	4.6			4.6			5.9			2.9		
frost-free days	88			48			78			28		
degreehours	10866			13635			21985			5881		
in degreedays	453			568			916			245		

Table 2. Climatic characteristics of measuring sites at contrasting elevations. Temperature was recorded hourly in the soil (10 cm depth), on the surface and in the air (10 cm above surface) from August 2011 to August 2012. July was the warmest month, January the coldest. Growing season is taken as a period with daily mean temperature above 0 °C (first-to-last day/ days actually). Degreehours (above 0 °C) were counted from hourly temperature recordings.

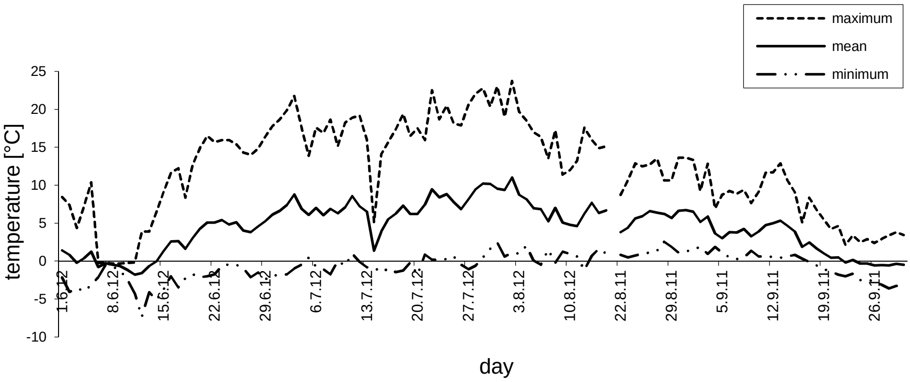

Fig. 8. Maximum, minimum and mean temperature of soil (10 cm depth) at 5820 m a.s.l. during vegetation season. Note that the data are combined from two years and for better comprehension the year 2012 precedes the year 2011. The measuring period lasted from August 2011 to August 2012

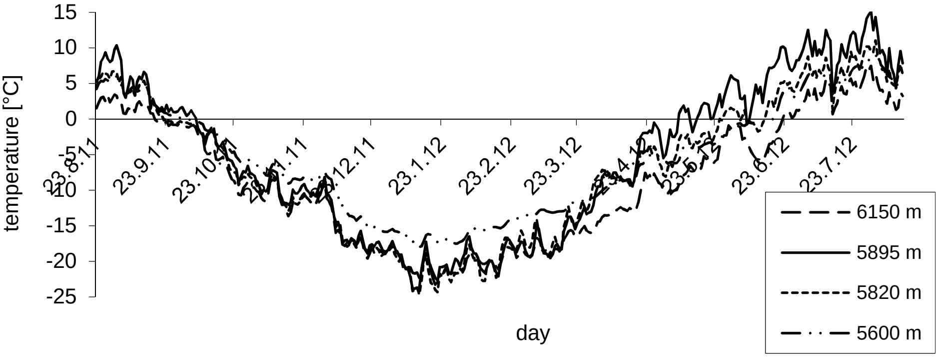

Fig. 9. Mean soil temperature at four elevational sites (5600 m, 5820 m, 5895 m, 6150 m). Temperature was recorded hourly at 10 cm depth from August 2011 to August 2012

period 2010–2011 (Online resource 4). The warmest site was on the eastern slope (the logger in the south collapsed), followed by the northern and western slopes (mean soil T of the warmest month 9.3, 6.6 and 5.9 °C, respectively). The sites were different also in the number of days when the soil did not freeze (eastern 84, nothern 58, western 67).

Our five-year long measurement (August 2008 to June 2013) of air T at 5900 m was probably too short to catch any significant trend (mean T was -11.6 °C).

Above 5800 m practically all precipitation falls as snow and it can occur all year round. The snowfall is only a sprinkle usually (0–3 cm) and melts the same day it falls enduring only a few hours in the sun. Based on RH as a proxy for snowfall, there were 22 snowfalls from July to September in 2009, 41 in 2010, 20 in 2011 and 28 in 2012. Continuous snow cover usually lies from January but with great differences between years (Fig. 10). The total number of days with either a snowfall or with a continous snow cover was 273 in 2009, 228 in 2010, 132 in 2011 and 141 in 2012. Lower winter mean T seemed to coincide with relatively stable and long snow cover. Snow cover during winter in 2008/09, 2009/2010 and 2012/13 lasted for at least four months continually, even if the beginning or the end could differ by months. These three winters were the coldest within the five seasons with mean T -16.6, -16.8 and -17.4 °C, respectively (measured from October to May). Snow cover in seasons 2010/11 and 2011/12 never lasted more than a month and a half continually and these winters had the highest mean T (-16.0 and -15.8 °C, respectively, Online resource 5).

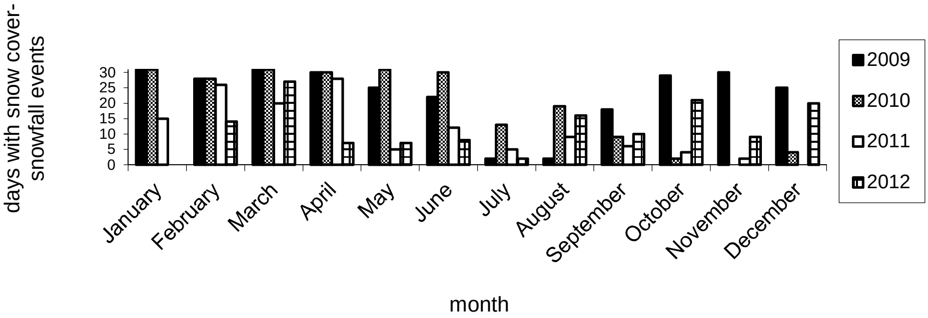

Fig. 10. Extrapolated numbers of days with snow cover/snowfall event in 5900 m in years 2009-2012. For extrapolation, automatic camera and a climatic datalogger were used to correlate snowfall events with air humidity values (see Methods for details). Missing bar means zero days with snow in the respective month (November 2010, December 2011, January 2012)

Growing season length

Our estimation of the growing season length is based on the concept of first-to-last day with the mean soil T > 0 °C (Table 2). Growing season defined in such a way lasted between 88 days (6150 m) and 153 (5895 m) with considerable portion of frost-free days (Table 2). Mean T of the soil ranged between 2.9 and 5.9 °C during growing season. The heat accumulation ranged between 245 and 916 degree days (in 6150 m and 5895 m, respectively). Two middle sites were rather similar in their T course (July to September 2012) with most frequent values between -1 and 4 °C. Temperatures between -1 and 1 °C dominated the highest site while the most frequent values at the lowest site varied between 3 and 7 °C (Fig. 11).

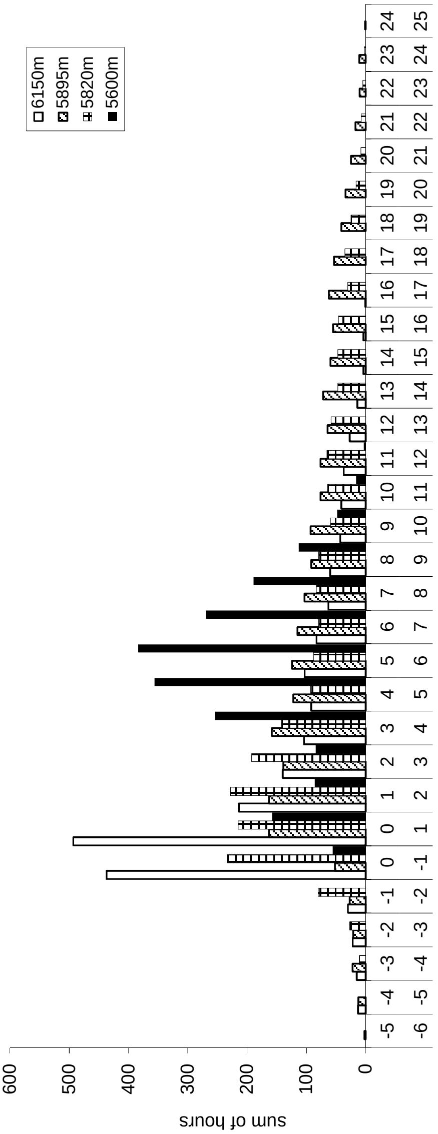

Fig. 11. Distribution of soil temperatures at contrasting elevations during growing season. Sum of hours with temperatures from respective ranges is shown. The temperature was recorded hourly at 10 cm depth from July to September 2012 at four elevational sites (5600 m, 5820 m, 5895 m, 6150 m)

Discussion

Delimitation of subnival zone

The calculation of nivality index based on the species composition of permanent plots (Gottfried et al. 2011) helped us to recognize the alpine-subnival ecotone. The result supported our assumption based on field observation that the ecotone lies at about 5700 m where the value of the index equalled 0.5 (approximated by quadratic trend line, $R^2 = 0.52$, Fig. 12). However, the calculation of the index is based on the classification of species (alpine or subnival) and this proved a little tricky in our study region, since many of them had unclear or wide centre of distributon (Fig. 7). No matter where it begins, the subnival vegetation zone has its core above 5800 m. From a floristic point of view, it is delimited by the absence of steppe species and by the presence of subnival specialists (10 species of 24). The remaining 14 species can be found in a wide range of elevation and some of them reach the very outpost of angiosperms at the same time (*Draba altaica*, *Poa attenuata*, *Saussurea* spp., *Stellaria decumbens*, *Waldheimia tridactylites*).

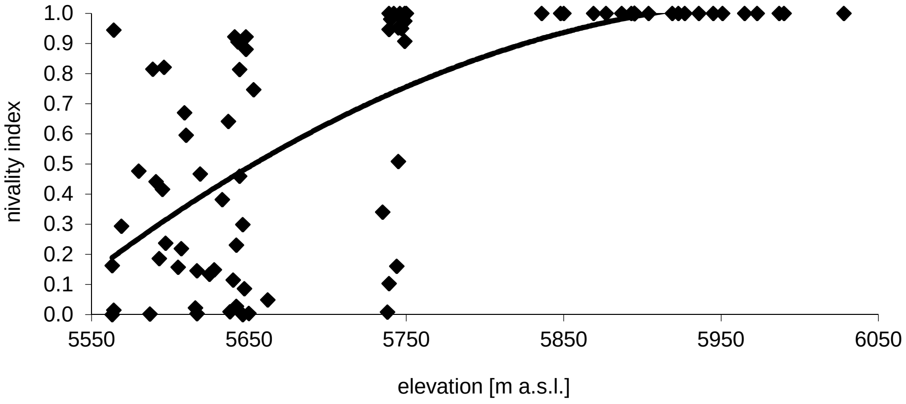

Fig. 12. Nivality index of permanent plots showing the ratio of subnival species to alpine species (sensu Gottfried et al. 2011) approximated by quadratic trend line (n = 91, $R^2 = 0.52$). The value of the index equalled 0.5 at about 5700 m, indicating the position of alpine-subnival ecotone

Importance of microclimate

The key factor for the existence of angiosperms at high elevations is the specific favourable microenvironment rather than elevation as such (Körner 2003), as was shown by the great microclimatic differences between our measuring sites. This was also illustrated experimentally by Klimeš and Doležal (2011) who showed that soil T at a site in 6160 m on a southwestern slope of Chalung Peak dropped below zero every day even during the short vegetation season. No higher plants were found by them at that site and the transplanted plants failed to survive; the elevation of 6160 m was concluded to be above the habitable zone of angiosperms within the region. At our site at 6150 m (cca 20 km south of Chalung Peak, southwestern slope) the soil was frost-free on 28 days and hosted 5 angiosperm species. Favourable soil temperature regime is vital for plants near the upper limit as the root tissue is more vulnerable to damage caused by ice formation in comparison to above-ground parts (Neuner and Hacker 2012).

Even if we did not quantify the separate effects of exposure and the specific microtopography, our results are in accordance with conclusions by Scherrer and Körner (2010; 2011) who stressed the importance of the thermal mosaic across topographically heterogenous alpine landscapes. In a multiscale study of soil T, they found substantial variation dependent on slope exposure (2–3 °C), microtopography within slopes (3–4 °C) and plant cover (1 °C within 1m^2 plots). In conclusion, elevation as such can be used only as a rough proxy of regional limits, explaining only a small part of a complex story.

Microsite differences result mainly from diverse exposure to radiation, wind and snow cover. Not only stones, but also cushion plants can be the objects providing shelter. Besides sheltering against wind these relatively dark objects absorb radiation and snow often thaws earlier in their close neighbourhood. As the summer snowfall is common in our study region, the advantage from growing near stones or cushion plants may be vital. Cushion plants were often shown to have ameliorative effect and to facilitate other species under harsh conditions (Arredondo-Núñez et al. 2009). However, *Thylacospermum caespitosum,* one of the most prominent cushion plants of the Tibetan Plateau, also common in our study region, did not show any facilitative effect (de Bello et al. 2012; Dvorský et al. 2013).

Coldest places with angiosperms

Angiosperm life from supposedly the coldest place on earth was reported by Körner (2011) from Swiss Alps. *Saxifraga oppositifolia* from a site at 4505 m needed for persistence 66 days of mean T of the rooting zone >0 °C (seasonal mean 2.6 °C) and the heat sum of 178 degree days (threshold 0 °C). The plant moreover experienced frost every day including the rooting zone. This record withstood comparison with vegetated sites from High Arctic (Svalbard) or Himalaya (5960 m) which were both warmer, and it also withstood comparison to our sites (Table 2). The coldest site in our study (6150 m), offered 88 days of mean soil T >0 °C (seasonal mean 2.9 °C) and 245 degree days. In addition, 28 days were without frost in the rooting zone.

Thus, the microsite properties including the growing season duration were apparently sufficient for at least the vegetative persistence of the plants. Such period of mere survival or vegetative growth can be of considerable length, as was documented by a herbochronological study of the species found at the highest site (6150 m). *Ladakiella klimesii* was found to be 22 years old and other four species 4 to 9 years old (Doležal unpublished data). These plants must have established during an exceptionally mild season which ensured successful germination and survival of the seedling (see also Körner 2011).

But what about the generative reproduction? Most studies on the reproductive development were done on Alpine species and the whole process from the break of dormancy to the seed maturation was documented to take 5–6 weeks with above freezing temperatures at the least (Wagner et al. 2012). All our sites offered more than that, even in number of days with mean T of soil >3 °C (not shown) while the thermal limit for growth of cold adapted plants lies about 2–3 °C (Körner 2003). Recent studies indicate that seed histogenesis may remain constant irrespective of the increasing sum of heat (Ladinig and Wagner 2005, 2007; Larl and Wagner 2006; Wagner et al. 2012). Another question is to what degree the seed development is slowed down by unfavourable events like cold spells or snowfall, usual in our study region. Frequency of such hours when plants stop growing or even get damaged seems to be of importance as for the total time needed for seed development and also for the reproductive success as such (Ladinig and Wagner 2007; Wagner et al. 2012; Ladinig et al. 2013).

Our discovery of vegetated patch at 6150 m represents a reliable record with its elevation measured several times both by GPS and altimeter, thus leaving little doubt about its correctness. This does not have to be the case of records traded for decades from the wet Nepalese Himalaya, e.g. *Saussurea gnaphalodes* from 6400 m, *Ermania himalayensis* from 6300 m, *Arenaria bryophylla* from 6200 m or *Stellaria decumbens* from 6100 m (Miehe 1991). Thus, it would be interesting to confirm these records with modern devices and to try to find plants at even higher elevations and assess their microenvironment. Based on our field experience, we expect such records in dry inner lands north of the main Himalaya Range, e.g. E Ladakh or W Tibet.

Limitations

In spite of the generally low cover and patchiness, we can consider the vegetation more or less continuous up to about 5950 m, i.e. it takes relatively little effort to discover a plant here. Higher up, vegetation patches get more and more isolated, above 6000 m plants need to be strenuously searched-for. Even if suitable microhabitats do exist this high, as can be seen from the vegetated site at 6150 m, they are much scarcer because the terrain turns steeper as the plateau lifts into the peaks and scree begins to dominate at about 6000 m. Thus the habitat limitation caused by terrain morphology and microsite limitation caused by severe climate play the key roles (for definitions see Münzbergová and Herben 2005). Seed, dispersal and recruitment limitations also seem to be important constraining factors in the population dynamics and colonization process. This idea is supported by our transplant experiment with surviving species (Chlumská, unpublished data). The sparse vegetation produces only a limited amount of ripe seeds, which only rarely get to a suitable microsite where another critical phase follows, the recruitment of seedlings. Species occurring above 6000 m belonged to four families (Brassiaceae, Asteraceae, Caryophyllaceae, Poaceae). In Asteraceae, the fruits are achenes with pappus which enables them to travel long distances no matter how tiny the maternal plant is; their seeds can be effectively brought from lower elevations by wind. The other six species have seeds with no appendages and the chance of a long distance transport from the seed source seems limited.

Therefore, the sufficient growing season length together with dispersal limitation supports our assumption that the species do reproduce generatively at the highest elevations here, at least some plants from the population and not necessarily every year (in 2011, *W. tridactylites* at 6150 m had flowers and we also found two loose Asteraceae seeds there). In order to test this assumption, germination experiments and seed trapping must be applied.

Possible effects of climate change

In the light of the ongoing climate changes we can expect both increasing temperature and rainfall in the nothern India. Mean annual T over the Asian land mass, including the Himalaya, is estimated to increase by about 5 °C and mean annual precipitation by 10–30 % by 2080 (Kumar et al. 2006; Treydte et al. 2006; IPCC 2007). The rise of temperature is well documented over the past decades (Shrestha et al. 1999; Liu and Chen 2000; Xu et al. 2009; Shrestha et al. 2012; Yue et al. 2013) and rates Himalaya among regions most vulnerable to warming. During the last 25 years, mean annual T has increased by 1.5 °C and the mean annual precipitation has increased by 163 mm in the Himalayan region

(Shrestha et al. 2012). Since 1960, mean annual temperature in the neighbouring Tibetan Plateau has increased by 1.91 °C with a warming rate of 443 % (Yue et al. 2013). Mean annual precipitation increased by 52.55 mm with increasing rate of 13 % and had continuously increasing trend, unlike other parts of China, where the trend alternated between increasing and decreasing decade by decade (Yue et al. 2013).

The climate change, however, differs among regions and seasons. The ecoregion of Western Himalayan alpine scrub and meadows, the nearest one to our study region, experienced significant warming in all four seasons (greatest warming in spring and winter) and a significant yearly rise in precipitation, mostly due to summer increase (Shrestha et al. 2012). These changes have already had effect on the growing season which tends to start earlier (Shrestha et al. 2012). On the other hand, the increasing winter temperature may have an adverse effect on the chilling requirements of plants, causing delayed spring phenology, as was documented for Tibetan steppe vegetation (Yu at al. 2010).

Plants growing at extreme altitudes are adapted to multiple stresses and some species are even restricted to the extreme conditions of subnival zone. This pose a threat on these specialised species, as they could be outcompeted by alpine species migrating from lower elevations under the scenario of rising temperatures (Gottfried et al. 2012, but see Scherrer et al. 2011). In eastern Ladakh, however, there seems to be enough space for upward-migrating species (unlike e.g. in the Alps), as the current limit of angiosperms is around 6150 m and the unglaciated peaks reach above 6400 m. Moreover, as was shown by Scherrer and Körner (2010, 2011), the microscale habitat differentiation very likely buffers the effect of climate warming on plants, thus providing the subnival species a suitable microclimate in their close surroudings with no need of upward migration. Our preliminary results based on repeated recording on permanent plots, however, showed no changes in vegetation structure and composition after four years (Dvorský unpublished data). Unfortunately, there are no studies on vegetation shifts from the region. Nevertheless, a year by year since the start of our project in 2008 we noticed greener slopes in Ladakh in general, as well as Hartmann mentioned even 15 years earlier (Hartmann 2009).

On the other hand, we hypothesize about other scenario. Since the existence of angiosperms at the upper limit here is possible thanks to snow- and glacier-free substrates (arid climate), the projected increase in precipitation could shift the snowline to lower elevation. The subnival zone would move downwards and it is a question whether the specialised species, some of them occurring in narrow altitudinal belts only (e.g. several *Draba* species), could follow this shift. Moreover, wet substrates are more susceptible to frost heave, a factor complicating seedling establishment. Indeed, the winter season 2012/13 brought the most extreme snowfall to eastern Ladakh in the last 50 years and caused heavy losses on herds, burrying the forage (personal communication with local people, news in media). Usually, the snow layer reaches several centimetres, but this time it reached nearly one meter. On the other hand, winter precipitation is highly unpredictable in Tibet and winter forage availability seems to control stock numbers (Goldstein et al. 1990, Miller 2005). Such a snowfall on traditional winter pastures with low snowfall risk may eventually fall within a normal range of this unpredictability, being highlighted by media just because of the losses on economically important pashmina goats. Notions of climate change, however, are also supported by recent extreme climatic events in the area of the capital city of Ladakh (Thayyen et al. 2013).

We found that the subnival vegetation up to its elevational limit is a mixture of specialized species (confined utterly to this environment) and species with broader niches (well-represented also in lower vegetation zones). This is an important finding with a conservational value. Given the impacts of climate change on the high-altitude vegetation all over the world (Gottfried et al. 2012), we conclude that the species in our study region are not threatened equally as there are apparently great differences in their ecological tolerance. Similar conclusion was made for adjacent alpine communities of Tibetan highlands by Miehe et al. (2011) who noted wide thermal and hygric range of many key species and considered this arid alpine biome resilient to climatic changes.

Supporting information

Additional supporting information may be found in the online version of this article:

Online resource 1 Photographs from automatic cameras focused on the slope with dataloggers and permanent plots (a). The red arrow on the first photograph points at the place with datalogger at 5900 m. Note the correlation between a snowfall event and relative air humidity (b)

Online resource 2 List of full names of species found above 5600 m a.s.l. Asterisk indicate species found only once above 5600 m

Online resource 3 Climatic characteristics for lower sites recorded from August 2011 to August 2012. Asterisk indicates a probably wrong value as the logger was overturned

Online resource 4 Climatic characteristics from three differently exposed slopes (north, west, east) of a site at 5895 m. Temperature was recorded hourly in the soil (10 cm depth), on the surface and in the air (10 cm above surface) from August 2010 to August 2011

Online resource 5 Air temperature and relative humidity at 5900 m a.s.l. in 2008-2013

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CHAPTER VIII
Summary of results

Photo on previous page: species-rich alpine grassland in southern Zanskar with striking plants of *Pleurospermum candollei* (Apiaceae).

Summary of results

The main objective of this thesis is to contribute to the exploration of Ladakh in the fields of botany and plant ecology. Focused on the least explored parts near the border with Tibet, this thesis provides insight into the main vegetation types and zonation, clonality in plants, plant-plant interactions and soil phototroph communities.

We distinguished eight main vegetation types in E Ladakh. The most important environmental factors influencing the species composition were elevation, soil moisture and salinity. Screes and alpine grasslands were found to be the most species-rich. However, with regard to vascular plant diversity, Ladakh can be concluded as “a cold spot”. It is no wonder, considering the harsh environmental conditions and geologic history (Chapter II).

Detailed inspection of plants and their below-ground organs enabled us to develop a system of clonal and growth form categories. Due to its hierarchical nature, this classification system is widely applicable in other mountain regions and enables comparison. We tested if the respective growth form categories reflected different habitat requirements, i.e. if they have adaptive value. Our results partly support this idea, because the proportion of some types of clonal growth forms in certain habitats was significantly higher. Some of the key functions can be highlighted, e.g. environment amelioration by cushion plants, search for water in plants with a perennial main root, anchorage in unstable substrates in plants with a pleiocorm having long branches, fast colonization of sandy and gravel deposits along rivers by horizontal roots with adventitious buds, and space occupancy by long hypogeogenous rhizomes in wetlands. I encourage the proposed preferences to be tested in other alpine regions of the world (Chapter III).

Another issue within our scope, plant-plant interactions along the gradient of stress, brought rather provocative results, which, however, fit well to the latest proposal of stress-gradient hypothesis. We showed that cushion plants, which have so far been overwhelmingly concluded as having positive (facilitative) effect on other plants, can behave competitively again above a certain limit of stress. We do not know precisely yet what is the true cause of the resulting negative interaction between cushion plants and other plant species. Indeed, it may be the result of the stronger competitive ability of cushions under conditions the cushions are better adapted to, but the cushions may just cease to provide any advantage for other plants. Anyhow, under combination of extreme elevation and aridity, no positive interactions between plants were detectable. Since similar results were brought by two our studies based on different experimental design, they represent valuable support for the emerging theoretical prediction of restricted facilitative effects under extreme conditions.

This evidence suggests that cases of missing strong facilitation are likely to be found in other extreme alpine conditions (Chapters IV and V).

The enquiry in soil phototroph communities and their structure revealed increasing proportion of cyanobacterial biomass with elevation and acknowledged the importance of biological soil crusts in nitrogen fixation. The importance of soil phototrophs on the establishment of vascular plants and their persistence, through various ways of improving substrate quality, however, remains to be assessed (Chapter VI).

Our last study brings description of one the highest-growing plant community on earth. It focuses on the traits of species and the microclimatic characteristics of habitats they occupy. Besides being purely descriptive in most parts, the study also brings review of the latest climatic prognoses for the region and hypothesizes about the climate-driven consequences for the plants at the upper elevational limit (Chapter VII).

APPENDIX

Coming from the south, the monsoon precipitation is stopped by the Himalaya Range – while southern slopes receive one of the highest amounts of rain in the world, lands north of it are arid (flight from Leh to New Delhi).

Southern slopes of the Himalaya Range near Rothang La Pass in Himachal Pradesh. Precipititation amount of about 1300 mm year^{-1} enables the existence of forests and lush alpine vegetation (highway from Manali to Leh).

Alpine meadows with *Geranium himalayense* (Geraniaceae) in Himachal Pradesh, 4200 m a.s.l.

Carpets of *Bistorta affinis* (Polygonaceae) occur in relatively wetter regions of Zanskar.
S Ladakh, 4800 m a.s.l.

Alpine meadow with *Pedicularis cheilanthifolia* (Orobanchaceae) in Zanskar region.
S Ladakh, 4900 m a.s.l.

Alpine grasslands with dominant *Carex* and *Kobresia* species in Nubra Valley, E Karakorum Range, N Ladakh, 5300 m a.s.l. Herds of domestic yaks graze here during summer.

A retreating glacier in a side valley of Nubra Valley, E Karakorum Range, N Ladakh, 5600 m a.s.l.

Old and freshly deglaciated moraines provide an ideal place for studying successional stages of vegetation. One of the pioneer species is *Carex nivalis* (Cyperaceae). Nubra Valley, E Karakorum Range, N Ladakh, 5500 m a.s.l.

Scree vegetation with *Delphinium brunonianum*, *Urtica hyperborea* and *Oxytropis microphylla.* Rupshu region, E Ladakh, 5200 m a.s.l.

Stabilized wet scree with exceptionally species-rich vegetation. Rupshu region, E Ladakh, 5400 m a.s.l.

Plains covered by cold steppes in Rupshu region, E Ladakh, 5300 m a.s.l. Characteristic vegetation components are *Stipa*, *Artemisia*, *Oxytropis, Carex, Kobresia and Elymus*. Local nomads use this region as winter pastures for their herds of goats and sheep. Note that the annual amount of precipitation is about 100 mm here.

Artemisia stracheyi (Asteraceae), a Himalayan endemic, is rare but locally common in steppe vegetation in Rupshu region, E Ladakh, 4800 m a.s.l.

Vegetation of gravelly plains with *Leymus secalinus, Ptilotrichum canescens* and *Carex moorcroftii.*
Rupshu region, E Ladakh, 4700 m a.s.l.

Cold steppe vegetation with *Saussurea glanduligera* (Asteraceae).
Rupshu region, E Ladakh, 4900 m a.s.l.

Shrubs of *Caragana versicolor* (Fabaceae) can dominate over vast areas.
Rupshu region, E Ladakh, 4600 m a.s.l.

Uprooting of shrubs for fuel by local nomads.
Korzok Village, Rupshu region, E Ladakh.

Hippophae rhamnoides ssp. *turkestanica* (Eleagnaceae) colonizes alluvial deposits of major rivers and provides berries rich in vitamins.

Stands of *Myricaria elegans* (Tamaricaceae) can be 3–5 m tall and form the local tree-line. Nubra Valley, N Ladakh, 4400 m a.s.l.

Brackish endorheic lake of Tso Moriri.
Rupshu region, E Ladakh, 4600 m a.s.l.

Wetlands around Tso Moriri lake are a protected Ramsar site with the only breeding ground
of Bar-headed goose (*Anser indicus*) in India.

Astragalus hendersonii (Fabaceae) is a rare species of upper alpine zone.
Rupshu region, E Ladakh, 5500 m a.s.l.

Desideria himalayensis (Brassicaceae) is a species of scree vegetation of upper alpine zone.
Stok Range, C Ladakh, 5500 m a.s.l.

Nepeta longibracteata (Lamiaceae) is a medicinal plant common in alpine screes.
Nubra Valley, E Karakorum Range, N Ladakh, 5300 m a.s.l.

Saussurea medusa (Asteraceae) - this distinctive species of Subgen. *Eriocoryne* is widespread, though apparently uncommon throughout the highest (granite) ranges of the Tibetan Plateau. Rupshu region, E Ladakh, 5600 m a.s.l.

A cushion of *Waldheimia nivea* (Asteraceae).
Nubra Valley, E Karakorum Range, N Ladakh, 5200 m.

Subnival vegetation with cushions of *Thylacospermum caespitosum* (Caryophyllaceae).
Rupshu region, E Ladakh, 5800 m a.s.l.

Arenaria bryophylla (Caryophyllaceae) and *Potentilla pamirica* (Rosaceae) are species typical for upper alpine vegetation. Rupshu region, E Ladakh, 5500 m a.s.l.

Ladakiella klimesii (Brassicaceae) is a subnival species discovered by Leoš Klimeš and later described and named in his honour. Rupshu region, E Ladakh, 6000 m a.s.l.

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