J. Mt. Sci. (2020) 17(3): 602-613 e-mail: jms@imde.ac.cn http://jms.imde.ac.cn
https://doi.org/10.1007/s11629-019-5386-y

Changes in regeneration and leaf traits of *Rhododendron campanulatum* along a treeline ecotone in central Nepal

SHARMA Prasad Krishna[1,2] https://orcid.org/0000-0002-3307-938X; e-mail: krishnaprasadsharmaacharya@gmail.com
TIWARI Achyut[1] https://orcid.org/0000-0001-9095-4067; e-mail: achyutone@gmail.com
SHRESTHA Babu Bharat[1*] https://orcid.org/0000-0002-9457-2637; e-mail: shresthabb@gmail.com

* *Corresponding author*

1 Central Department of Botany, Tribhuvan University, Kathmandu, 44613, Nepal

2 Budhanilkantha School, Kathmandu, 44622, Nepal

Citation: Sharma PK, Tiwari A, Shrestha BB (2020) Changes in regeneration and leaf traits of *Rhododendron campanulatum* along a treeline ecotone in central Nepal. Journal of Mountain Science 17(3). https://doi.org/10.1007/s11629-019-5386-y

Abstract: Changes in population structure and plant functional traits across environmental gradient in the near natural treeline is a critical determinant of environmental changes in treelines. In this study we analyzed population characteristics and leaf traits of *Rhododendron campanulatum* across treeline ecotone in a trans-Himalayan region of Mustang district in central Nepal. Along 4 vertical transects, a total of 72 quadrats (5 m × 5 m) - 34 above *Betula utilis* treeline and 38 below treeline - were sampled to analyze the population structure, regeneration, specific leaf area (SLA) and leaf-stem relationship of *R. campanulatum*. Our results showed that the regeneration of *R. campanulatum* was fairly good in both habitats, as evident from density-diameter curve. The species showed type III survivorship curve in both habitats with the highest mortality during early stage of life. However, the seedling density and mortality was higher below treeline than above treeline. Canopy cover and basal cover were found to be the major factors affecting regeneration, as indicated by higher number of juveniles in relatively open areas above treeline. The total leaf area increased ($p < 0.001$) with stem cross-section area in both habitats. However, the SLA was higher below treeline (55 cm^2/g) than above (47 cm^2/g) ($p < 0.001$); it also increased with the canopy cover of trees ($R^2 = 0.59$, $p < 0.001$) below the treeline. In conclusion, high density of juvenile, low mortality of seedlings, and low SLA above treeline indicated that the species has successfully adapted in climatically stressful alpine habitat with potential to expand upward.

Keywords: *Rhododendron***;** Climate change; Life table; Mortality; Specific leaf area; Mustang district

Received: 08-Mar-2019
Revised: 08-Sep-2019
Accepted: 11-Oct-2019

Introduction

Many studies dealing with demography of trees found that treeline species showed increased seedling and juvenile density, improved growth rate with better adaptation at higher elevations in response to warming temperature (Hofgaard 1997; Körner 1999; Harsch et al. 2009; Gaire et al. 2016; Tiwari et al. 2016). The demographic response of plant population deals with statistics of recruitment, mortality, population structure, regeneration, and this is the major tool to predict the stability and trends of plant population in future (Harper and White 1974; Forbis 2003; Lauenroth and Edler 2008). In response to warming temperature, increase in density of tree species in treeline ecotone is anticipated prior to upslope shift of treeline (Rauh 1978; Hofgaard 1997). The key stages of population development can provide the population structure and viability of species in the particular community which ultimately shows the regeneration patterns (Boyce 1992).

Plants growing in higher elevations are adapted to low temperature with decreasing height and specific leaf area (Paulsen et al. 2000; Westoby and Wright 2003). Decreasing diameter and height with increasing elevation are dominantly caused by the corresponding decline in temperature (Schickhoff 2005). At cooler temperatures production of adenosine triphosphate (ATP) is low and photosynthetic products are transferred to sugar and starch rather than cellulose that limit the entire growth of plants (Holtmeier 2009; Tranquillini 2012). Small plant height is beneficial because it experiences high ground temperature during growing season (Körner 1999). In addition, thick bark and high root-to-shoot ratio in mountain plants enable them to tolerate low temperature and resource scarcity (Mooney and Billings 1960; Scott and Billings 1964; Sakai and Larcher 1987; Frejaville et al. 2013). However tree growth in higher elevation could also be decreased by warming induced seasonal drought (Cook et al. 2003; Dawadi et al. 2013; Liang et al. 2014; Qi et al. 2015; Gaire et al. 2016; Tiwari et al. 2016).

Climatic treeline ecotones are ecological zone of transition marked by a change in site conditions and vegetation which are mostly considered as indicators for the study of landscape response to global climatic change (Holtmeier 2009; Körner 2012; Tranquillini 2012). Among the climatic factors, temperature is the most influencing factor of alpine treeline that limits the growth and regeneration of species (Körner, 1999; Holtmeier and Broll 2007; Harsch et al. 2009). Low temperature in the sub-alpine forest resulted in less number of tree and shrub species; this is the reason for significantly high importance value index (IVI) of few species (Lei et al. 2002; Shrestha et al. 2007) and these few dominant species form continuous population along elevation gradient.

Rhododendron (Ericaceae) is an important genus of mountain vegetation in Nepal Himalaya which can be found from *ca.* 1500 m asl with subtropical climate to >5000 m asl with nival climate (Milleville 2002). Life form of this genus also ranges from a tall tree (*Rhododendron arboreum* Sm., height reaching up to 16 m) to a miniature shrub (*Rhododendron nivale* Hook. f., height 15-30 cm). In spite of diversity and socio-economic importance only a few studies have examined the regeneration and functional traits of this taxon (e.g. Ranjitkar et al. 2013; Schwab et al. 2016; Bürzle et al. 2018). Among 32 species of *Rhododendron* found in Nepal Himalaya (Rajbhandari and Watson 2005); *Rhododendron campanulatum* D. Don. (Vernacular name: Chimal) constitutes an important element in treeline ecotone (Schwab et al. 2016, 2017). It is a shrub or sub-tree which extends its distribution from 2800 m to 4400 m asl from Kashmir to southeast Tibet and all over the Nepal Himalaya except in some dry areas of Trans-Himalayan regions (Polunin and Stainton 1997). It is a major understory component of sub-alpine forest forming pure stand above treeline but sometimes associated with Junipers, *Rhododendron campylocarpum* Hook.f. etc. In lower elevations it is erect attaining height up to 8 m and stem diameter to 30 cm but towards upper limit the plant is creeping with short stature (Milleville 2002; Ohba et al. 2008).

A few studies have examined the population structure and regeneration of *R. campanulatum* on the southern declivity of the Himalaya in eastern and central Nepal (e.g. Schwab et al. 2016; Rana et al. 2017). In Rolwaling valley in eastern Nepal, Schwab et al. (2016) reported a good regeneration of this species (inverse J –shaped density diameter distribution) with very high density of recruits (<7 cm diameter at breast height) in treeline ecotone (5434-6360 stem/ha) compared to the closed-canopy stands far below treeline (960 stem/ha). Another study showed an increasing density of younger individuals of *R. campanulatum* with substantial expansion to higher elevation in Manaslu Conservation Area of central Nepal (Rana et al. 2017). However, the population structure and regeneration of this species is not known in the Trans-Himalayan region with rain shadow effect. Furthermore, none of the earlier studies has analyzed the changes in leaf traits of this species across treeline ecotone. Since the pattern of plant functional trait changes along environmental gradient can be a reliable predictor of community response to climate change (Guittar et al. 2016), analysis of plant functional traits along with population characteristics can give better insight than studying the population characteristic alone. Therefore, we aimed to 1) compare population characteristics (population structure and regeneration) and leaf traits of *R. campanulatum* above and below the treeline, and 2) understand adaptive changes of population characteristics and leaf traits in the contrasting habitats of treeline ecotone. For this study, we selected the north-east facing treeline ecotone of Mustang district in central Nepal.

1 Materials and Methods

1.1 Study site

The study area (Lat. 28.613°-28.600°N, Long. 083.580°-083.593°E) is located at Mustang district (Kaligandaki river valley) of Annapurna Conservation Area in north-central Nepal (Figure 1a). Mean annual precipitation is 1390 mm (at 2384 m asl, Lete station) and mean annual temperature is 11.28°C (DHM 2013). Some small glaciers are present at higher elevations predominantly on north facing slopes. The Kaligandaki river valley is situated at *ca*. 2700 m asl, and mountain peak reaches upto 8167 m asl (Mount Dhaulagiri -the seventh highest mountain in the world).

Betula utilis D. Don forms diffuse treeline (*sensu* Harsch et al. 2009) in the study area. This area is usually covered with snow during winter season (November to April). In the lower parts (2000 to 3000 m asl) *Pinus wallichiana* A.B. Jacks and *Tsuga dumosa* (D.Don) Eichler forest is mixed with other broad leaved species such as *Aesculus indica* (Wall. ex Cambess.) Hook., *Juglans regia* L., *Rhododendron arboreum* etc. *Abies spectabilis* (D.Don) Mirb. and *B. utilis* forests are dominant between 3000 to 3700 m asl, and the understory is dominated by *R. campanulatum* around treeline (~3700 m asl) followed by *Sorbus microphylla* (Wall. ex Hook.f.) Wenz. *R. campanulatum* was present as continuous treeline vegetation on moist sites. Above treeline, alpine tundra vegetation was dominated by dwarf shrubs; these include *R. campanulatum*, *R. anthopogon* D.Don and *Cassiope fastigiata* (Wall.) D.Don. Medicinal plants such as *Dactylorhiza hatagirea* (D.Don) Soo.

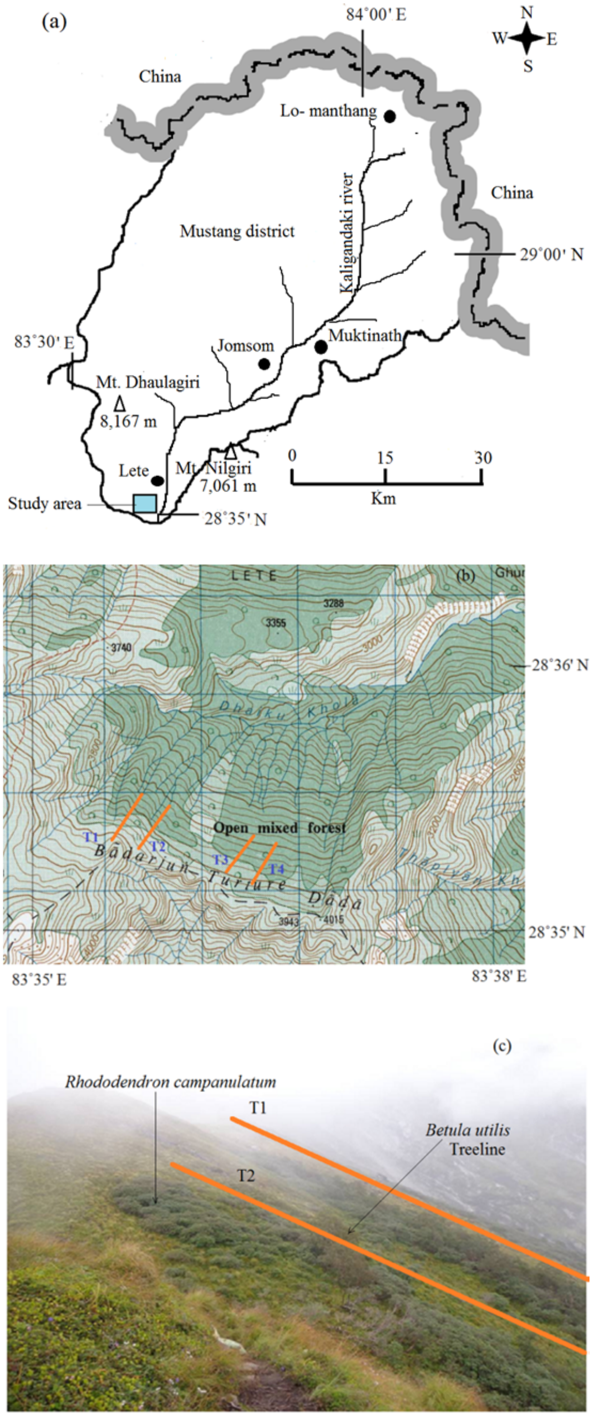

Figure 1 Geographical location of the study area; Mustang district, Nepal (a), contour map (each contour = 40 m) with transects (b). Close photograph of treeline ecotone and segment of transects with position and direction (c).

(Protected species in Nepal), *Neopicrorhiza scrophularifrora* (Pennell) DY Hong., *Nardostachys grandiflora* DC. were also present in the alpine meadow (3500-4000 m asl). The area has been influenced by various human activities such as grazing, extraction of fuel wood, and medicinal plant collections (including *Ophiocordyceps sinensis* (Berk.) G.H. Sung, J.M. Sung, Hywel-Jones & Spatafora) and those activities are usual in summer season (May-October).

1.2 Field sampling and lab works

Field sampling was conducted during monsoon season in September 2014. Additional information such as photographs, size of the smallest fruiting individual of *R. campanulatum*, and lower elevation limit of the species were collected in 2015 in the same season. Four vertical elevation transects (T1-T4) were established at horizontal distance of at least 300 m intervals with treeline position at 3953 m, 3920 m, 3775 m and 3984 m asl, respectively (Figure 1b, c). Slightly depressed treeline position of third transect (3775 m asl) was possibly due to its' position in windward side (Holtmeier 2009). In these vertical transects altogether 72 plots (5m×5m) were sampled between 3700 m to 4070 m asl. The sampled sites lie between 28.61°N-28.59°N latitude and 083.58°E-083.59°E longitude with slope between 9° and 35°. All the sampled transects were in the North-East aspect because other sites were inaccessible. In each transect 18 plots (8 above and 10 below treeline in T1 and T2 and 9 each above and below treeline in T3 and T4) were sampled. Less number of plots above treeline in T1 and T2 was due to the less expansion of the species above treeline than in other transects (T3 and T4). The surface distance between adjacent plots was 15 m, and a buffer zone 7.5 m was left in each side just above and below the treeline. The treeline was determined according to Körner (1999) which was the upper limit of tree species with a dominant stem that reaches a height of at least three meters (≥3 m). Elevation, aspect and slope were measured by using Altimeter and Clinometer, respectively. Highly disturbed and rocky places with steep slope (>35°) were avoided from sampling which resulted to unequal length of transects.

In each sampling plot, all individuals of *R. campanulatum* were recorded. The individuals less than 20 cm height were considered as seedlings, individuals with height >20 cm but basal diameter (at 20 cm above ground) <4 cm were considered as juvenile because the smallest fruiting individual recorded had 4 cm basal diameter. Individuals with >4 cm basal diameter were considered as adults. Basal diameter of adults was measured at 20 cm above the ground by using diameter tape. Canopy cover of tall trees (*Betula utilis, Abies spectabilis*) was recorded by visual method.

Leaf and twig samples of *R. campanulatum* were collected for leaf trait analysis from 67 plots; suitable individual for sampling were absent in the remaining 5 plots. During sampling, a healthy adult individual was selected from each plot and a twig containing at least three healthy mature leaves were sampled from sun facing side. Diameter of the sampled twig was also measured just below the ultimate whorl of leaves by vernier caliper (0-150 mm) to analyze the relationship between stem cross-section area and total leaf area. Length (excluding petiole) and breadth at the widest part of all fresh leaves were measured. Among these leaves, outlines of 30 leaves (15 from above treeline and 15 from below treeline) were drawn in A4 size paper (70 GSM; Paper One Company Ltd. Indonesia). The sampled leaves were kept in between newspaper using herbarium press and brought to lab for the determination of dry mass following Cornelissen et al. (2003). The leaves were kept in hot air oven at 80°C for 72 hours and weighed using digital balance (0.001 g).

1.3 Data analysis

Population of *R. campanulatum* was divided into seedling, juvenile and adult (Table 1). Adults were further categorized to the basal diameter size classes of 4-<8 cm, 8-<12 cm, 12-<16 cm and so on.

Table 1 Density of different life stages of *R. campanulatum* above and below treeline

Life stages	Density (stem/ha)		*p
	Above treeline	Below treeline	
Seedlings	$1176^{a} \pm 3.36$	$3968^{b} \pm 1.13$	0.00029
Juveniles	$482^{a} \pm 1.12$	$389^{b} \pm 1.17$	0.032
Adults	$1800^{a} \pm 2.30$	$2969^{b} \pm 3.88$	0.034
Total	$3458^{a} \pm 3.34$	$7326^{b} \pm 3.93$	0.0001

*p values are based on Z-test. The mean values sharing different superscript letters in each row indicates the significant difference.

Basal cover of *R. campanulatum* in the sampling plot was calculated by adding the basal cover of all individuals present in the plot and was expressed as the percentage of the area sampled. Based on population structure, life table and survivorship curve were prepared following Zhang et al. (2008). In life tables (Table 2), a_x = total number the survival individuals; l_x =proportion of individuals surviving from the beginning to size class x or $l_x = (a_x/a_o)\times1000$; d_x = number of dead individuals from size class x to $x+1$ or $d_x = a_x - a_{x+1}$; q_x = mortality from x to $x+1$, or $q_x = d_x/ l_x$; L_x = the mean number of survival individuals from size class x to x+1 or $L_x = (l_x + l_{x+1})/2$; T_x = Total number of survival individuals from size class x or $T_x = \sum L_x$, and e_x is the life expectancy at size class x or $e_x = T_x/l_x$. Mortality (q_x) and life expectancy (e_x) of each size class were determined from life tables. The paper cut-out of the 30 leaves was weighed to calculate area of each leaf (Cornelissen et al. 2003). A correction factor was determined using following relations: Correction factor = leaf area/(length × width). The leaf area correction factor for above the treeline was 0.7402 ± 0.0063 (Mean ± SD) and for below the treeline was 0.729 ± 0.00385. Since there was no significant difference in two correction factors (t-test, df= 28, and p = 0.129), their mean was used for further analysis. For rest of the leaves, the area was obtained by multiplying the product of length and width by the correction factor obtained as above. For each twig, total leaf area was obtained as the sum of area of all the leaves. Twig diameter was used to calculate stem cross section area. The specific leaf area (SLA) of each leaf was obtained as the ratio of leaf area to dry mass (Cornelissen et al. 2003).

The data were tested for normality (Shapiro-Wilk test; alpha level 0.05) before performing statistical analyses. The SLA and stem cross section area were square root and log transformed, respectively, in order to meet the assumption of normality. The values of stem basal cover did not meet the assumption of normality. Therefore, regression was not performed and only scattered diagrams have been presented to show the variation of density of seedling and juvenile with stem basal cover. Densities of seedling, juvenile and adult above and below treeline were compared by Z-test. Similarly, area of individual leaf and the SLA of above and below treeline were also compared by Z-test. Variation of the SLA with tree canopy cover and that of total leaf area with stem cross section area were analyzed by linear regression. All statistical analyses (e.g. t-test, Z-test, regression etc.) were carried out using Statistical Package for Social Science (SPSS, version 16.0.0).

2 Results

2.1. Population structure and regeneration

Rhododendron campanulatum was present in all sample plots above and below treeline. Seedling,

Table 2 Life table of *R. campanulatum* above and below treeline. See method section for description of each variable calculated.

	Size class (cm)	a_x	l_x	d_x	q_x	L_x	T_x	e_x
Above treeline	Seedlings	1176	1000	590.13	0.59	704.93	2271.25	2.27
	Juveniles	482	409.86	-170.91	-0.41	495.32	1566.32	3.82
	4-<8	683	580.78	290.81	0.50	435.37	1071.01	1.84
	8-<12	341	289.96	19.55	0.06	280.18	635.62	2.19
	12-<16	318	270.40	140.30	0.51	200.25	904.45	3.34
	16-<20	153	130.10	79.93	0.61	90.13	155.18	1.19
	20-<24	59	50.17	10.20	0.20	45.06	65.05	1.29
	24-<28	47	39.96	39.96	1	19.98	19.98	0.5
	>28	0	0	0	0	0	0	0
Below treeline	Seedlings	3968	1000	901.97	0.90	549.01	1344.01	1.34
	Juveniles	389	98.03	-114.16	-1.16	155.11	794.98	8.11
	4-<8	842	212.19	-34.52	-0.16	229.46	639.86	3.02
	8-<12	979	246.72	148.43	0.60	172.50	410.41	1.66
	12-<16	390	98.28	-8.06	-0.08	102.31	237.90	2.42
	16-<20	422	106.35	61.23	0.57	75.73	135.58	1.27
	20-<24	179	45.11	15.87	0.35	37.17	59.85	1.32
	24-<28	116	29.23	21.16	0.72	18.64	22.68	0.77
	>28	32	8.06	8.06	1	4.03	4.03	0.5

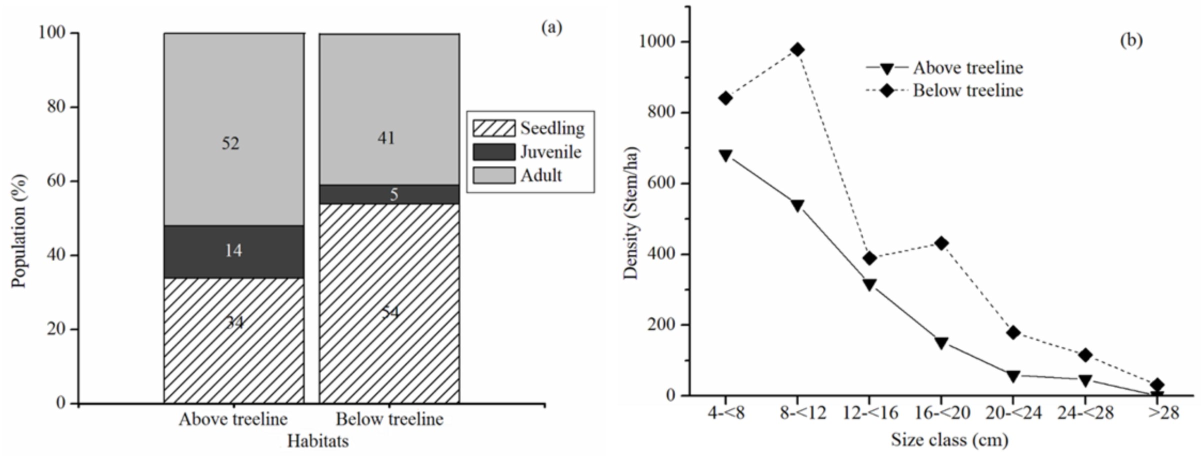

Figure 2 Population structure of *R. campanulatum;* Relative population of major life stages based on Table 1 (a) and Density-diameter curve of adults (b).

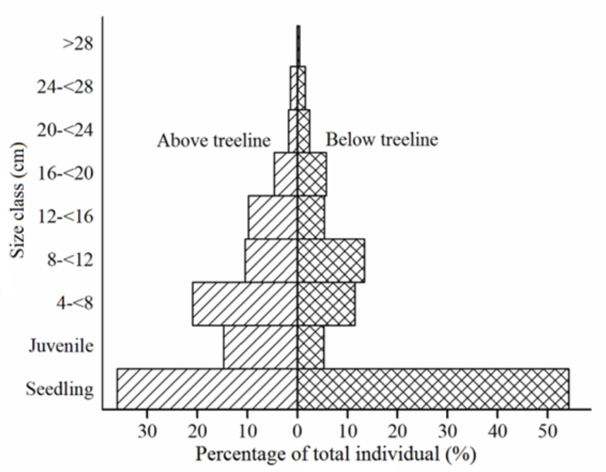

Figure 3 Population pyramids of *R. campanulatum* above and below treeline.

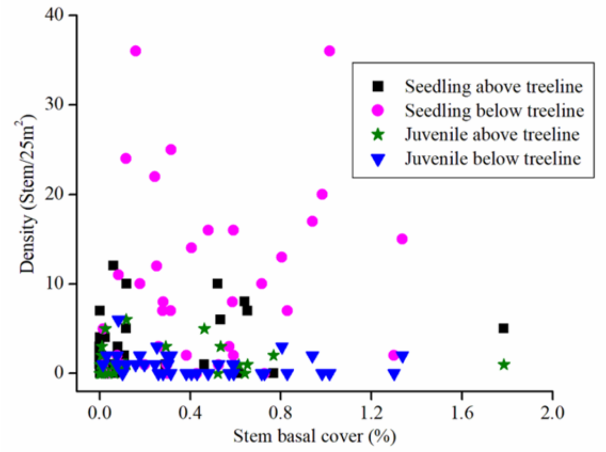

Figure 4 Scatter diagrams showing the variation of seedling and juvenile density of *R. campanulatum* with basal cover.

juvenile and adult were present in 70%, 100% and 60% sample plots, respectively above treeline, and 90%, 85% and 100% sample plots, respectively, below treeline. In the population of *R. campanulatum*, the proportion of juvenile and adult were higher above treeline than that of below treeline but the proportion of seedling was higher below treeline than of above (Figure 2a). Density of seedling and adult below treeline were 3.37 and 1.65 times higher (p= 0.00029 and 0.034, Z-test), respectively, than above treeline but the density of juvenile was 1.24 times higher above treeline than below treeline (p= 0.032, Z-test) (Table 1).

The density-diameter (d-d) curves of adult *R. campanulatum* showed a general decline in density with increasing diameter class, suggesting a good regeneration in both habitats (Figure 2b). However, the density was higher below treeline than above for all size classes. Population pyramids also showed more individuals in lower size class (seedling) and decreasing towards higher size classes in both habitats (Figure 3). Population structure of above treeline habitat was relatively stable than below treeline, as evident from the higher percentage of individuals in the intermediate size classes above treeline compared to below treeline. Stem basal cover of the species was 0.185% and 0.445% in above and below the treeline, respectively. There was no significant effect of stem basal cover on the density of seedlings and juveniles of *R. campanulatum* both above and below treeline (Figure 4). However, seedling and juvenile were almost absent below treeline when basal cover reaches >1.3%. But above

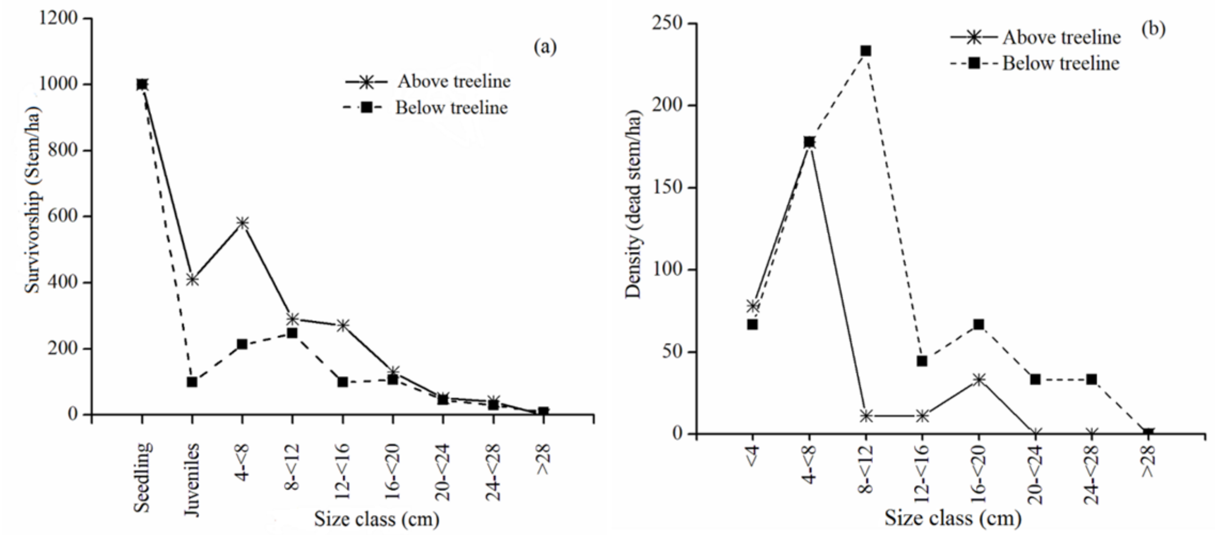

Figure 5 Survivorship (lx) curves based on life tables (Table 2) (a) and stem mortality in field condition (b) of *R. campanulatum*.

treeline, seedling and juvenile were present in the plots having basal cover even up to 1.83 %.

2.2 Mortality and survivorship curve

Mortality (q_x) of seedling was 59% above treeline and 90% below treeline (Table 2), which became the first death peak during the population development. In both habitats, the juveniles exhibited the highest life expectancy (e_x).

The survivorship curve of *R. campanulatum* in both habitats was close to Deevey-III (*sensu* Deevey 1947) (Figure 5a), which showed a high mortality of seedlings with only 41 % and 10% survival above and below treeline, respectively. The mortality of all size classes was lower (i.e. high survivorship) above the treeline than below. Dead stem density of *R. campanulatum* was higher below treeline in all size classes (Figure 5b).

2.3 Leaf characters

Area of the individual leaf of *R. campanulatum* was higher below treeline than of above treeline (46.08 cm^2 ± 1.38 vs. 38.85 cm^2 ± 1.30; p = 0.035; Z-test). The mean specific leaf area (SLA) of *R. campanulatum* was 51.44 ± 1.09 cm^2/g and it was higher below treeline (54.67 ± 1.37 cm^2/g) than above (47.05 ± 1.42 cm^2/g) (p = 0.0003; Z-test). Below treeline, the SLA of *R. campanulatum* increased (p <0.001) with increasing canopy cover of trees (Figure 6). Total leaf area supported by a stem increased (p <0.001) with increasing stem cross-section area in both habitats; however, the increase in leaf area per unit increment of stem cross section area was lower above treeline than below (Figure 7).

3 Discussion

Population structure of *Rhododendron campanulatum* across treeline ecotone revealed that the regeneration potential of this species was higher above treeline than below, with the possibility of further upslope shift in response to

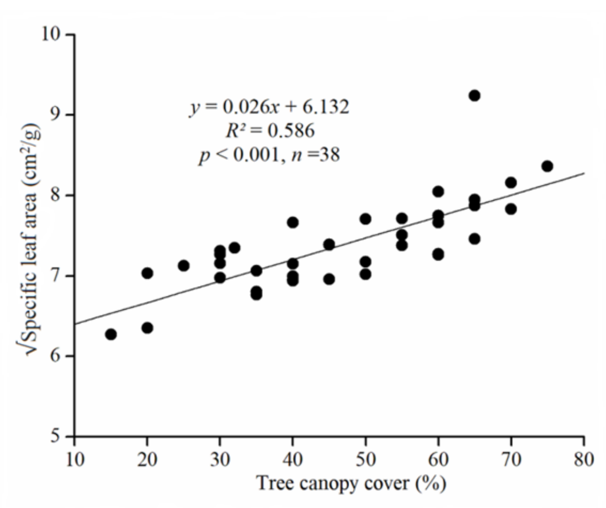

Figure 6 Regression analysis showing variation in specific leaf area (SLA) of *R. campanulatum* with tree canopy cover below treeline.

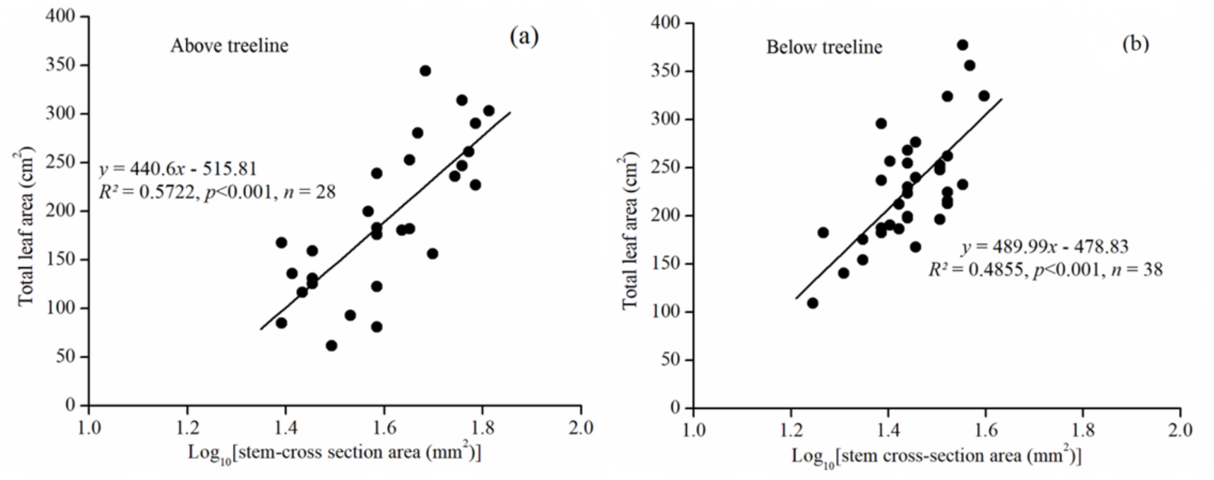

Figure 7 Regression plots showing variation in total leaf area of *R. campanulatum* with stem cross section area above treeline (a) and below treeline (b). (Note higher slope of the fitted line for below treeline than for above treeline).

climate warming. Higher density of juvenile above treeline compared to below treeline clearly showed the potentially higher regeneration of *R. campanulatum* in alpine habitat near treeline. This was also corroborated by lower mortality of seedlings above treeline (59%) than below (90%). The higher regeneration of *R. campanulatum* above treeline and relatively low regeneration below treeline was also reported in the treeline ecotone of Manaslu Conservation Area in central Nepal (Rana et al. 2017). Dense krummholz of this species has been found to have controlled the upslope migration of other tree species (Schwab et al. 2016). Increase in density of individuals of tree species (i.e. densification) is likely to precede upslope shift of treeline in response to climate warming (Rauh 1978; Hofgaard 1997). A similar densification of tree species in treeline ecotone in response to climate warming has been also anticipated in eastern Himalaya (Sikkim, India) (Pandey et al. 2018). These processes may lead to the expansion of woody vegetation at the cost of grasslands in alpine habitats. The encroachment of shrubs in the grasslands changes the structure and functioning of ecosystem and facilitates for the further encroachment (Eldridge et al. 2011). These encroachment processes in the grassland are also considered as the part of successional process and decreasing disturbance patterns (Sharma et al. 2014).

Plant regeneration and survival in treeline ecotone is often constrained by low temperature and snow characteristics (Whitmore 1989; Tranquillini 2012). Ongoing global warming is, however, expected to alleviate this temperature constraint at present day treeline ecotone (Körner 1999; Harsch et al. 2009). In addition to climate, biotic interaction is also an important factor affecting regeneration in treeline ecotone (Liang et al. 2016; Schwab et al. 2017). Density of particular species in alpine life can greatly be enhanced by neighboring plant species in the community (Choler et al. 2001) but they are dependent mostly in life histories and stress factor (Maestre et al. 2009).

Population pyramid of *R. campanulatum* showed an expanding nature of its population with significant number of individuals in all size classes. However, the population pyramid of *R. campanulatum* below treeline revealed a very high density of seedlings but the individuals in other size classes were very low. This kind of population structure has been also reported in central Himalayan *Quercus floribunda* Lindl. ex A. Camus and *Pinus roxburghii* Sarg. (Saxena and Singh 1984). Disproportionately high density of seedling compared to larger size classes indicates frequent reproduction but low survival of seedlings. The high seedling mortality of *R. campanulatum* can be attributed to high light interception by tree canopy below treeline (Marchand and Chabot 1978; Lei et al. 2002; Castro et al. 2004; Shrestha et al. 2007). The density of seedling and juvenile individuals of *R. campanulatum* in the study site were higher in relatively open area but did not show any significant relationship with the stem basal cover.

However seedling establishment of *R. campanulatum* in a Himalayan treeline ecotone was found to be favored by bryophyte mats with mean site cover 80% (Bürzle et al. 2018). Some studies also suggested that seedling mortality in the higher elevations is often correlated to the low temperature and drought during cold winter that lead to the high mortality of seedlings in the relatively open area (Marchand and Chabot 1978; Coates 2002; Castro et al. 2004). However, our data revealed that seedling mortality of *R. campanulatum* was lower in relatively open area, i.e. above the treeline (Figure 4, Table 2). Due to presence of tree canopy, light reaching to ground vegetation is low below the treeline than above where trees are absent. But the ambient temperature is expected to be higher below treeline than above. Therefore, our data suggests that seedling mortality may be more dependent to light than to low temperature within the current treeline ecotone region. However, this assertion needs further evaluation by monitoring individual seedling in field experiments.

Regeneration of *R. campanulatum* might have been affected by the occurrence of dwarf shrub *R. anthopogon* above treeline. The patches of *R. anthopogon* may provide safe and warm environment for the seedling recruitment of *R. campanulatum,* in a way similar to the report of Cavieres and Badano (2009) in high Andes. This facilitative role of *R. anthopogon* could be a reason for more frequent occurrence of *R. campanulatum* seedling and juvenile within *R. Anthopogon* patches than in open sites observed during sampling. Bürzle et al. (2018) has also emphasized the positive role of safe sites for successful regeneration of *R. campanulatum* in the Himalayan treeline ecotone. Furthermore, other factors such as stand conditions (e.g. basal cover, stems density, canopy gap), wild animals, pathogens, human disturbance, etc., regulate the plant regeneration in the sub-alpine and alpine regions (Whitmore 1989; Singh and Singh 1992; Tang and Zhai 2004; Tranquillini 2012).

According to the local people and our field observations the treeline of study site was disturbed by two human activities; grazing and the medicinal plant collection. The above treeline habitat was mainly disturbed by livestock grazing while the below treeline forest was disturbed by humans during firewood collection. Selection of *R. campanulatum* as the best and easily harvestable firewood could be a reason for the unusual density-diameter curve for below treeline. These anthropogenic disturbances are considered the major factors to control the plant regeneration (West et al. 1981) and woody plants are likely to be affected more by such disturbances than other life forms (Evju et al. 2012). However, in disturbed sites around treeline some woody species such as *Rhododendron* can regenerate fairly high (Saxena and Singh 1984).

Life tables provide the information on the mortality of population to predict the future population structure of the stand (Zhang et al. 2008). The survivorship curve of *R. campanulatum* prepared from life table was close to Deevey-III with a peak of mortality in the stage of seedling. Life expectancy reached the peak at juvenile's stage in both habitats above and below treeline (Table 2). In general species which follow the survivorship curve of type III are called r-strategists (Pianka 1970; Singh et al. 2006). *R. campanulatum* showed some characters of r-strategies such as small seed size, pioneer in succession and follow the type III survivorship curve. On the other hand it showed some characters of k-strategists such as density-dependent mortality, slow growth, and long life span (Singh et al. 2006). Due to these common characters of r-strategists and k-strategists, *R. campanulatum* therefore can be categorized as stress tolerant (Grime 2006). Hence, mixed life strategy, higher proportion of juvenile and adult, and lower seedling mortality above treeline showed that the species has been successfully adapted to the environmental conditions above treeline.

Leaf traits of *R. campanulatum* measured in the present study across treeline ecotone showed that the plant can adjust them according to the environmental conditions. Below treeline, *R. campanulatum* grows as understory element where light intensity as well as evapotranspiration is generally lower but temperature is higher than above treeline (Coates 2002; Castro et al. 2004). Leaf number, leaf size, twig length and diameter are the key parameters for the study of plant adaptation in the particular habitat (Sun et al. 2006). These parameters generally decrease with increasing elevation and environmental stresses.

With larger individual leaves, higher increase in leaf area per unit increment of stem cross sectional area, and higher SLA below treeline, the species can maximize carbon assimilation (Lambers and Poorter 1992; Wang et al. 2017). Plants are generally adapted by increasing the leaf size in order to capture the diffused light from relatively larger area. This assertion is also supported by the increase in SLA of *R. campanulatum* with increasing tree canopy cover in the present study. Above treeline, the environmental condition is more extreme with high evapotranspiration and low temperature associated with high solar radiation. Under such conditions, survival and persistence is more important than maximizing carbon assimilation (Körner 2012). The reduced SLA above treeline favors the species to survive in the climatically stressful conditions by reducing transpiration (Garnier et al. 2001). Low SLA leaves tend to have longer leaf lifespan, presumably because stronger leaf reinforcement is necessary to assure the longer life. In most cases SLA of a particular plant species depends on the elevation (temperature), moisture, light and soil nutrients (Poorter and Jong 1999; Garnier et al. 2001). Plants in higher elevation of sub-alpine and alpine regions are generally adapted by decreasing their leaf size, leading to low SLA (Westoby and Wright 2003; Luo et al. 2005).

4 Conclusions

Success of *Rhododendron campanulatum* in different habitats with contrasting microclimatic conditions can be attributed to adaptive variation of population characteristics and leaf traits. High density of juvenile with low mortality of seedling even under the environmentally stressful habitat above treeline indicated that *R. campanulatum* is successfully adapted above treeline and can potentially persist the harsh environmental conditions there. In addition, small leaves with low SLA can increase stress tolerance to survive under stressful environment above treeline. Similarly the higher density of juveniles above treeline could be the sign of upslope migration. The improved regeneration in response to increasing global temperature will contribute in expansion of species gradually upslope and may alter the mountain ecosystem and negatively affect livelihood of the mountain communities as the upward expansion may decrease the pasture area used for grazing. However, further studies on growth-climate relationship, species interaction and succession process, specific impacts of different disturbance factors would be required to validate above predictions. And, the interactions between climate and land use change patterns are equally critical in understanding the population dynamics and physiological mechanism of *R. camapulatum* in tree line ecotone of the Himalayan region.

Acknowledgements

This research work was supported by WWF Nepal under "The Generation Green" which was provided to first author (KPS) through Resources Himalaya Foundation Nepal under "Building next generation of conservation leaders 2015" MSc dissertation small grant. We are also grateful to Tribhuvan University, Central Department of Botany for the invaluable support by providing field equipment and lab facilities and to Mr. Raju Bista for his kind help in field work.

References

Boyce MS (1992) Population viability analysis. Annual Review of Ecology and Systematics 23(1): 481-506. https://doi.org/10.1146/annurev.ecolsys.23.1.481

Bürzle B, Schickhoff U, Schwab N, et al. (2018) Seedling recruitment and facilitation dependence on safe site characteristics in a Himalayan treeline ecotone. Plant Ecology 219(2): 115-132. https://doi.org/10.1007/s11258-017-0782-2

Castro J, Zamora R, Hódar JA, Gómez JM (2004) Seedlings establishment of a boreal tree species (*Pinus sylvestris*) at its southernmost distribution limit, consequences of being in a marginal Mediterranean area. Journal of Ecology 92(2): 266-277. https://doi.org/10.1111/j.0022-0477.2004.00870.x.

Cavieres LA, Badano EI (2009) Do facilitative interactions increase species richness at the entire community level? Journal of Ecology 97(6): 1181-1191. https://doi.org/10.1111/j.1365-2745.2009.01579.x

Choler P, Michalet R, Callaway RM (2001) Facilitation and competition on gradients in alpine plant communities. Ecology 82(12): 3295-3308. https://doi.org/10.1890/0012-9658(2001)082[3295:FACOGI]2.0.CO;2

Coates KD (2002) Tree recruitment in gaps of various sizes, clear cuts and undisturbed mixed forest of interior British Columbia, Canada. Forest Ecology and Management 155(1-3): 387-398. https://doi.org/10.1016/S0378-1127(01)00574-6

Cook ER, Krusic PJ, Jones PD (2003) Dendroclimatic signals in long tree-ring chronologies from the Himalayas of Nepal. International Journal of Climatology 23(7): 707-732. https://doi.org/10.1002/joc.911

Cornelissen JHC, Lavorel S, Garnier E, et al. (2003) A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Australian Journal of Botany 51(4): 335-380. https://doi.org/10.1071/BT02124

Dawadi B, Liang E , Tian L , et al. (2013) Pre-monsoon precipitation signal in tree rings of timberline *Betula utilis* in the central Himalayas. Quaternary International 283: 72–77. https://doi.org/10.1016/j.quaint.2012.05.039

Deevey ESJ (1947) Life tables for natural populations of animals. Quarterly Review of Biology 22(4): 283-314. https://doi.org/10.1086/395888

DHM (2013) Temperature and precipitation data of Nepal, Department of Hydrology and Meteorology, Government of Nepal, Kathmandu.

Eldridge DJ, Bowker MA, Maestre F, et al. (2011) Impacts of shrub encroachment on ecosystem structure and functioning: towards a global synthesis. Ecology Letters, 14(7): 709-722. https://doi.org/10.1111/j.1461-0248.2011.01630.x

Evju M, Hagen D, Hofgaard A (2012) Effects of disturbance on plant regrowth along snow pack gradients in alpine habitats. Plant Ecology 213(8): 1345-1355. https://doi.org/10.1007/s11258-012-0094-5

Forbis TA (2003) Seedling demography in an alpine ecosystem. American Journal of Botany 90(8): 1197-1206. https://doi.org/10.3732/ajb.90.8.1197

Frejaville T, Curt T, Carcaillet C (2013) Bark flammability as a fire-response trait for subalpine trees. Frontiers in plant science 4: 466. https://doi.org/10.3389/fpls.2013.00466

Gaire NP, Koirala M, Bhuju DR, Carrer M (2016) Site- and species-specific treeline responses to climatic variability in eastern Nepal Himalaya. Dendrochronologia 41: 44-56. https://doi.org/10.1016/j.dendro.2016.03.001

Garnier E, Shipley B, Roumet C, Laurent G (2001) A standardized protocol for the determination of specific leaf area and leaf dry matter content. Functional Ecology 15(5): 688-695. https://doi.org/10.1046/j.0269-8463.2001.00563.x

Guittar J, Goldberg D, Klanderud K, et al. (2016) Can trait patterns along gradients predict plant community responses to climate change? Ecology 97(10): 2791-2801. https://doi.org/10.1002/ecy.1500

Grime JP (2006) Plant Strategies, Vegetation Processes, and Ecosystem Properties. John Wiley & Sons, New Jersey, USA.

Harper J, White J (1974) The demography of plants. Annual Review of Ecology and Systematics, *5*(1): 419-463. https://doi.org/10.1146/annurev.es.05.110174.002223

Harsch M, Hulme PE, McGlone MS, Duncan RP (2009) Are treelines advancing? A global meta-analysis of treeline response to climate warming. Ecology Letters 12(10): 1040-1049. https://doi.org/10.1111/j.1461-0248.2009.01355.x

Hofgaard A (1997) Inter-relationships between treeline position, species diversity, land use and climate change in the central Scandes Mountains of Norway. Global Ecology and Biogeography Letters 6(6): 419-429. https://doi.org/10.2307/2997351

Holtmeier FK (2009) Mountain Timberlines: Ecology, Patchiness and Dynamics. Springer Science and Business Media, Berlin, Germany.

Holtmeier FK, Broll G (2007) Treeline advance - driving processes and adverse factors. Landscape Online 1: 1-33. https://doi.org/10.3097/LO.200701

Körner C (1999) Alpine Plant Life; Functional Plant Ecology of High Mountain Ecosystems. Springer Science and Business Media, Berlin, Germany.

Körner C (2012) Alpine Treelines: Functional Ecology of the Global High Elevation Tree Limits. Springer Science and Business Media, Berlin, Germany.

Lauenroth WK, Adler PB (2008) Demography of perennial grassland plants: survival, life expectancy and life span. Journal of Ecology, *96*(5): 1023-1032. https://doi.org/10.1111/j.1365-2745.2008.01415.x

Lei TT, Semones SW, Walker JF, et al. (2002) Effects of *Rhododendron maximum* thickets on tree seed dispersal, seedlings morphology, and survivorship. International Journal of Plant Sciences 163(6): 991-1000. https://doi.org/10.1086/342711

Liang E, Dawadi B, Pederson N, Eckstein D (2014) Is the growth of birch at the upper timberline in the Himalayas limited by moisture or by temperature? Ecology 95 (9): 140307191613003. https://doi.org/10.1890/13-1904.1

Liang E, Wang Y, Piao S, et al. (2016) Species interactions slow warming-induced upward shifts of treelines on the Tibetan Plateau. Proceedings of the National Academy of Sciences of the United States of America 113(16): 4380-4385. https://doi.org/10.1073/pnas.1520582113

Luo T, Luo J, Pan Y (2005) Leaf traits and associated ecosystem characteristics across subtropical and timberline forests in the Gongga Mountains, Eastern Tibetan Plateau. Oecologia 142(2): 261-273. https://doi.org/10.1007/s00442-004-1729-6

Maestre FT, Callaway RM, Valladares F, Lortie CJ (2009) Refining the stress - gradient hypothesis for competition and facilitation in plant communities. Journal of Ecology 97(2): 199-205. https://doi.org/10.1111/j.1365-2745.2008.01476.x

Marchand PJ, Chabot BF (1978) Winter water relations of tree-line plant species on Mt. Washington. Arctic and Alpine Research 10 (1): 105-116. https://doi.org/10.1080/00040851.1978.12003947

Milleville RD (2002) The Rhododendrons of Nepal. Himal Books, Kathmandu, Nepal.

Mooney HA and Billings WD (1960) The annual carbohydrate cycle of alpine plants as related to growth. American Journal of Botany 47: 594-598. https://doi.org/10.1002/j.1537-2197.1960.tb14911.x

Ōhba H, Iokawa Y, Śarmā L (2008) Flora of Mustang, Nepal. Kodansha Scientific Ltd, Tokyo.

Pandey A, Badola HK, Rai S, Singh SP (2018) Timberline structure and woody taxa regenerationtowards treeline along latitudinal gradients in Khangchendzonga National Park, Eastern Himalaya. PLoS ONE 13(11): e0207762. https://doi.org/10.1371/journal.pone.0207762

Paulsen J, Weber UM, Körner C (2000) Tree growth near treeline: Abrupt or gradual reduction with elevation? Arctic, Antarctic and Alpine Research32 (1): 14-20. https://doi.org/10.1080/15230430.2000.12003334

Pianka ER (1970) On r- and k- selection. American Naturalist 104(940): 592-597. https://doi.org/10.1086/282697

Polunin O, Stainton A (1997) Concise Flowers of Himalaya. Oxford University Press, India.

Poorter H, De Jong ROB (1999) A comparison of specific leaf area, chemical composition and leaf construction costs of field plants from 15 habitats differing in productivity. New Phytologist 143(1): 163-176. https://doi.org/10.1046/j.1469-8137.1999.00428.x

Qi Z, Liu H,Wu X, Hao Q (2015) Climate-driven speedup of alpine treeline forest growth in the Tianshan Mountains, Northwestern China. Global Change Biology 21 (2):816–826. https://doi.org/10.1111/gcb.12703

Rajbhandari KR, Watson M (2005) *Rhododendrons* of Nepal (Fascicle of Flora of Nepal, Volume 5, Number 4). Department of Plant Resources, Thapathali, Kathmandu, Nepal.

Rana P, Bhuju DR, Koirala M, Boonchird C (2017) Dendroecological studies of *Rhododendron campanulatum* D.Don. along the elevational gradient of Manaslu Conservation Area, Nepal Himalaya. Pakistan Journal of Botany 49(5): 1749-1755.

Ranjitkar S, Luedeling E, Shrestha KK, et al. (2013) Flowering phenology of tree *Rhododendron* along an elevation gradient in two sites in the Eastern Himalayas. International journal of biometeorology 57(2): 225-240. https://doi.org/10.1007/s00484-012-0548-4

Rauh W (1978) Die Wuchs- und Lebensformen der tropischenHochgebirgsregionen und der Subantarktis, ein Vergleich. In: Troll C and W Lauer (eds.), Geoecological Relations between the Southern Temperate Zone and the Tropical Mountains. Steiner, Wiesbaden. pp 62-92.

Sakai A, Larcher W (1987) Frost Survival of Plants: Responses and Adaptation to Freezing Stress. Ecological Studies, Vol. 62. Berlin: Springer. http//doi.org/10.1007/978-3-642-71745-1

Saxena AK, Singh JS (1984). Tree population structure of certain Himalayan forest associations and implications concerning future composition. Vegetation 58 (2): 307-324. https://doi.org/10.1007/BF00044928

Schickhoff U (2005) The upper timberline in the Himalayas, Hindu Kush and Karakorum: a review of geographical and ecological aspects. In: Broll G and B Keplin (eds.), Mountain Ecosystems: Studies in Treeline Ecology. Springer-Verlag, Berlin. pp 275-354.

Schwab N, Schickhoff U, Müller M, et al. (2016) Treeline Responsiveness to Climate Warming: Insights from a Krummholz Treeline in Rolwaling Himal, Nepal. In: Singh R, Schickhoff U, Mal S (eds.), Climate Change, Glacier Response, and Vegetation Dynamics in the Himalaya. Springer, Cham. pp 307-345. https://doi.org/10.1007/978-3-319-28977-9_16

Schwab N, Schickhoff U, Bürzle B, et al. (2017) Implications of tree species – environment relationships for the responsiveness of Himalayan krummholz treelines to climate change. Journal of Mountain Science 14(3): 453-473. https://doi.org/10.1007/s11629-016-4257-z

Scott D and Billings WD (1964) Effects of environmental factors on standing crop and productivity of an alpine tundra. Ecological Monograph 34(3): 243-270. https://doi.org/10.2307/1948502

Sharma LN, Vetaas OR, Chaudhary RP, Måren IE (2014). Ecological consequences of land use change: forest structure and regeneration across the forest-grassland ecotone in mountain pastures in Nepal. Journal of Mountain Science 11(4): 838-849.
..
https:// doi.org/ 10.1007/s11629-013-2849-4

Shrestha BB, Ghimire B, Lekhak HD, Jha PK (2007) Regeneration of treeline birch (*Betula utilis* D. Don) forest in a trans-Himalayan dry valley in central Nepal. Mountain Research and Development27 (3): 259-267.
https://doi.org/10.1659/mrdd.0784

Singh JS, Singh SP (1992) Forest of Himalaya. Gyanodaya Prakashan, Nainital, India

Singh JS, Singh SP, Gupta SR (2006) Ecology Environment and Resource Conservation. Anamaya Publishers, New Delhi. India

Sun S, Jin D, Shi P (2006) The leaf size–twig size spectrum of temperate woody species along an altitudinal gradient: an invariant allometric scaling relationship. Annals of Botany 97(1): 97-107. https://doi.org/10.1093/aob/mcj004

Tiwari A, Fan ZA, Jump AS, et al. (2016) Gradual expansion of moisture sensitive Abies spectabilis forest in the Trans-Himalayan zone of central Nepal associated with climate change. Dendrochronologia 41: 1-10. https://doi.org/10.1016/j.dendro.2016.01.006

Tranquillini W (2012) Physiological Ecology of the Alpine Timber Line: Tree Existence at High Elevations with Special Reference to the European Alps. Springer Science and Business Media, Berlin, Germany.

Wang M, Liu GH, Jin TT, et al. (2017) Age-related changes of leaf traits and stoichiometry in an alpine shrub(*Rhododendron agglutinatum*) along altitudinal gradient. Journal of Mountain Science 14(1). https://doi.org/10.1007/s11629-016-4096-y

Westoby M, Wright IJ (2003) The leaf size–twig size spectrum and its relationship to other important spectra of variation among species. Oecologia135 (4): 621-628. https://doi.org/10.1007/s00442-003-1231-6

Whitmore TC (1989) Canopy gaps and the two major groups of forest trees. Ecology 70 (3): 536-538. https://doi.org/10.2307/1940195

Zhang QY, Luo P, Zhang YC, et al. (2008) Ecological characteristics of *Abies georgei* population at timberline on the north facing slope of Baima Snow Mountain, Southwest China. Acta Ecologica Sinica 28(1): 129-135. https://doi.org/10.1016/S1872-2032(08)60022-0