



Regeneration and leaf traits variation of *Rhododendron campanulatum* along elevation gradient in western Nepal Himalaya

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ARTICLE INFO

Received January 22, 2024

Revised February 26, 2024

Accepted March 7, 2024

Published on April 23, 2024

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Background: Plant species of the alpine treeline ecotone are highly sensitive to climate change and may adjust their population dynamics, and functional traits in response to changing climate. This study examined regeneration patterns and leaf traits variations in an important treeline ecotone element *Rhododendron campanulatum* along the elevation gradient in western Nepal to assess its potential adaptive responses to climate change. The distribution range of *R. campanulatum* (3,400–3,800 m above sea level [a.s.l.]) was divided into five horizontal bands, each with a 100 m elevational range. Eight plots (10 m × 10 m) were sampled in each band, resulting into a total of 40 plots. In each plot, all *R. campanulatum* individuals and co-occurring tree species were counted. From each elevation, *R. campanulatum* leaf samples were collected to determine leaf dimensions, leaf density, specific leaf area (SLA), and stomatal density (SD).

Results: The density-diameter curve indicated that *R. campanulatum* was regenerating well, with enhanced regeneration at higher elevation (3,800 m a.s.l.) than at lower. Tree canopy cover appeared to be the major determinant of *R. campanulatum* regeneration, as indicated by a higher number of seedlings in treeless stands. With increasing elevation, the leaf length, width, SLA, and stomata length decreased but leaf thickness and SD increased.

Conclusions: Overall, a higher regeneration and lower SLA with the high SD in the leaves at the upper limit of the species distribution suggested that *R. campanulatum* is well adapted at its upper distribution range with the possibility of upslope range shift as temperature increases.

Keywords: climate change, leaf stomata, Nepal Himalaya, plant functional traits, specific leaf area, treeline ecotone

Introduction

High-elevation regions are particularly sensitive to shifting climatic belts due to global climate changes, and, consequently, they are strong indicators of climate change because the vegetation they have is highly influenced by temperatures (Grabherr et al. 1994). The growth and reproduction of plant communities in higher elevations are mainly controlled by temperature (Grace et al. 2002), resulting in steep ecological gradients along with elevation and restricted ecotone (Pauli et al. 2015). Therefore, minor fluctuations in ambient temperature may induce alterations in the elevational position of the treeline. Additionally, many plant species are shifting upward as a result of

global warming, creating higher stand densities and driving treelines to higher elevations (Gaire et al. 2017; Singh et al. 2018; Tiwari et al. 2017).

Treeline dynamics can be characterized by studying regeneration patterns of the treeline forming species (Mainali et al. 2020; Sharma et al. 2020), which may be reflected in the population structure (Tiwari et al. 2018). Successful regeneration is indicated by the presence of an adequate number of seedlings, saplings, and young trees in a given population (Mishra et al. 2013; Pokhriyal et al. 2010). Regeneration not only displays the current condition, health, and vitality of the forest but also shows how the forests will look like in the future (Zhang et al. 2007). Regeneration varies along elevation gradients due to differing tempera-



tures, precipitation, and vegetation zones. Climate change disrupts this process by modifying temperature and moisture levels, consequently disturbing critical phases such as flowering, germination, and seedling establishment of the plant species (Vandvik et al. 2020). Furthermore, climate change intensifies disturbances, like fires and pests, impacting the regenerative capacity of plants across various elevations (De Deus Vidal et al. 2021).

In addition to regeneration, plants also respond to elevation and climatic gradients by changing functional traits and other physiological processes. For example, the species that grow in cold temperature or at very high elevations can cope with stressful environmental conditions like low temperatures, intense radiation, less water availability, and strong winds by changing the characteristics of their leaves, such as by making them smaller and thicker (Liu et al. 2020), yet they are still sensitive to climate change (Zhang et al. 2010). As a result, leaf area (LA), as well as specific leaf area (SLA) decrease as elevation rises and temperature decline (Zhang et al. 2010). A reduction in leaf size and an increase in thickness can increase a plant's mechanical strength, allowing it to endure stressful environments like freezing temperatures (Lütz 2010). The variation in biological processes like growth, survival, and reproduction is known to be significantly influenced by leaf functional traits such as the LA, and SLA (Chen et al. 2021; Wright et al. 2004). Therefore, the leaves play a significant role in overall ecosystem functioning and are the most vulnerable organ to climate change in plants (Huang et al. 2020; Shi et al. 2020). Additionally, stomata—the turgor-operated valves to regulate the exchange of gases between plant tissues and the atmosphere—are crucial for controlling the cycling of both water and carbon (Taylor et al. 2012). Environmental changes may alter the form, distribution, and density of stomata (Hetherington and Woodward 2003), as higher stomatal density (SD) has been found with increased sunlight exposure (Kelly and Beerling 1995), rising elevation (Woodward 1986), and lower atmospheric CO₂ concentration (Royer 2001).

Responses of mountain plants to environmental gradients such as the elevation (a proxy measure of change in temperature and other climatic variables) may vary from one region to another. For example, a synthesis by Zobel and Singh (1997) has revealed that the Himalayan forests are functionally and structurally distinct both from tropical and temperate forests, and that biosphere-level ecological generalization with poor representation of Himalayan data may introduce multiple errors in such generalizations. Together with this, high vulnerability and sensitivity of the Himalayan vegetation to climate change warrants additional studies on functional and structural aspects of the forest in the Himalaya, a data poor region (Chakraborty et al. 2018; Zobel and Singh 1997). One of the major plants of high elevation vegetation in the Himalaya are *Rhododen-*

dron (Ericaceae) species. In Nepal, 31 species of *Rhododendron* are known to exist, with their distribution spanning from subtropical to nival regions (DoFSC 2019). Among these species, *Rhododendron campanulatum* D. Don thrives within the treeline ecotone of Nepal, which has been classified as one of the most vulnerable ecosystems in the world (Wielgolaski et al. 2017). The species plays an important role in the treeline ecosystem by enhancing soil fertility and stability and influencing the soil water dynamics by intercepting precipitation and reducing evaporation (Singh et al. 2019). It also shows high adaptability and resilience to the harsh climatic conditions and potential upward expansion due to global warming (Singh et al. 2018). A previous study has suggested that this species has already shifted upward away from the *Abies spectabilis* (D. Don) Spach treeline (Mainali et al. 2020), and that the species is well adapted in climatically stressful alpine habitat (Sharma et al. 2020). In another study, Schwab et al. (2017) have reported that krummholz formed by *R. campanulatum* has prevented other tree species from shifting upslope in response to climate change. Moreover, compared to the *Betula utilis* D. Don treeline, *R. campanulatum* exhibits a significantly more rapid process of stand densification and shifting to form a pure stand above the treeline (Tiwari and Jha 2018). Given that all above studies were conducted in central and eastern Nepal, the regeneration status and plant functional trait variation of *R. campanulatum* along the elevation gradients remain unknown in western Nepal. Therefore, we chose *R. campanulatum* as the target species of our study in western Nepal Himalaya, aiming to gain insight into its adaptive mechanisms and responses to changing environmental conditions. We aimed to 1) study changes in regeneration of *R. campanulatum* along the elevation gradient and 2) analyse the variation of leaf traits of *R. campanulatum* along the elevation gradient. Quadrat sampling method was used to analyse population structure and subsequently access regeneration. Selected leaf functional traits were determined in leaf samples collected from each quadrat. Studying the population structure of dominant species along elevation gradients in mountainous regions may provide valuable insights into how environmental factors affect the species' natural regeneration. By examining the functional characteristics of plants across elevation gradients, we can gain insights into how species are adapted to the harsh alpine environment and how they may respond to global climate change.

Materials and Methods

Study area

This study was conducted in the Khali forest of Kankasundari rural municipality (29.21°N latitude, 82.09°E longitude, elevation: 3,400 to 3,800 m above sea level [a.s.l.]), which is lo-

cated at the middle of the Sanja region of Jumla district in western Nepal (Fig. 1). The mean annual precipitation is 1,256 mm, and the mean annual temperature is 10.15°C (DHM 2017). About half (47%) of the district's area is covered by forest (Acharya and Paudel 2020). A reconnaissance survey of the study area has revealed a clear zonation of the forest types. In the lower parts (2,000 to 3,000 m a.s.l.), there are *Pinus wallichiana* A. B. Jacks. forests with broad-leaf species such as *Quercus semecarpifolia* Sm., *Juglans regia* L., and *Rhododendron arboreum* Sm. are major associated species. Between 3,000 and 3,800 m a.s.l., there are *A. spectabilis* forests and *B. utilis* forests. In the *B. utilis* forest, the understory vegetation is dominated by *R. campanulatum* which expands well above the *B. utilis* treeline. Therefore, *R. campanulatum* is a major element of the treeline ecotone.

Study species

Rhododendron campanulatum, also known as *Chimal* in Nepal, is a shrub, or small tree up to 8 m high (Singh et al. 2018) with reddish-brown or white stems and smooth bark that peels off in thin flakes. The leaves are shiny, leathery, arranged in whorls at the branch ends, oval to elliptical in shape, and around 9.5–14 cm long. The flowers, which are pale mauve to rosy-purple (sometimes white with purple spots), form loose clusters with rounded bell-

shaped corollas (Polunin and Stainton 1987). Flowering occurs during April–May, followed by fruiting in June (Bisht et al. 2014).

Vegetation sampling

The field sampling was conducted during August 2022. The population structure of *R. campanulatum* and associated tree species was studied by a systematic sampling method. In the study area, *R. campanulatum* was found from 3,400 to 3,800 m a.s.l. The forest was divided into five horizontal transects defined at an elevation range of 100 m and eight plots (10 m × 10 m) were sampled in each transect with a total of 40 plots throughout the elevations (Fig. 1). In each plot, all individuals of *R. campanulatum* and associated tree species were counted. Individuals with a height of ≥ 2 m were categorized as trees (Körner 2012). Individuals ≤ 20 cm tall were recorded as seedlings, whereas those with a height ≥ 20 cm and < 2 m were categorized as saplings (Sharma et al. 2020). The linear tape was used to measure the basal diameter of trees (measured at 20 cm above the ground) and the height of seedlings and saplings, while the basal diameter of seedlings and saplings was measured at 5 cm above the ground by a digital vernier caliper. Each plot was divided into four sub-plots (5 m × 5 m), and two sub-plots lying diagonally were selected randomly for seedling and sapling counts.

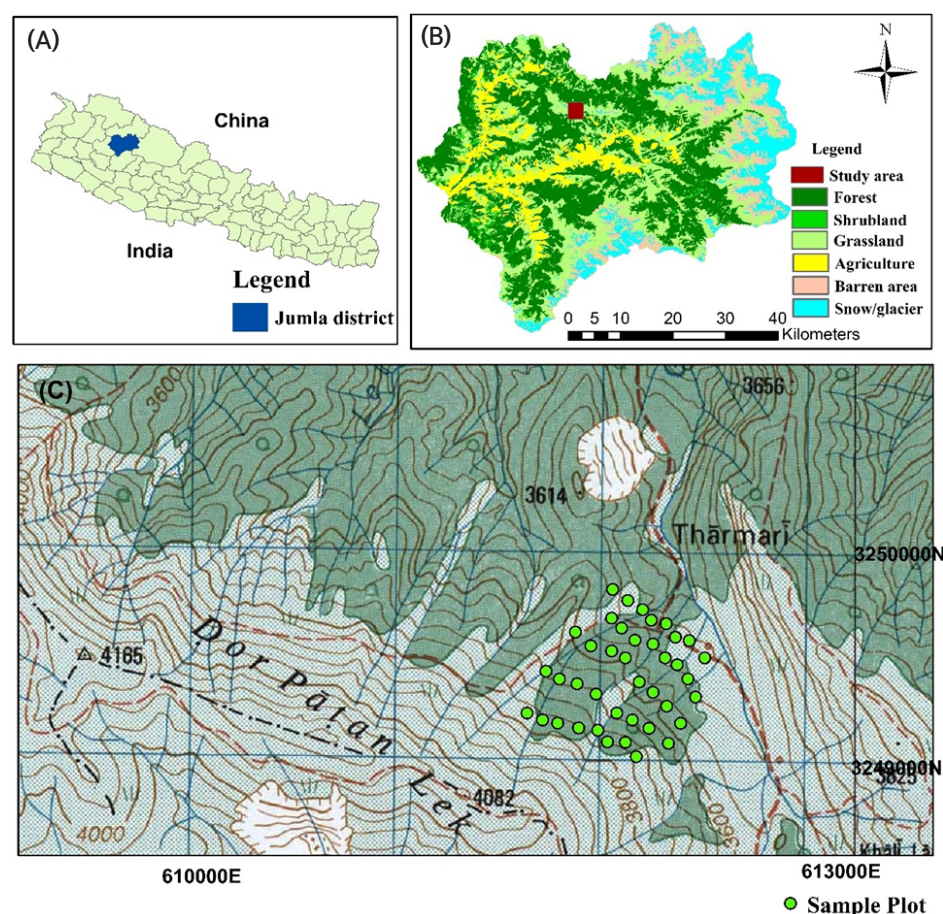


Fig. 1 Location of Jumla district (B) in Nepal (A) and location of plots in the study area (C).

Leaf morphological traits

During field sampling, five healthy adult plants with no sign of disease were selected in each quadrat. Five leaves fully exposed to sunlight were collected from each of the selected trees; altogether, 25 leaves were collected from each quadrat. The length, width, thickness, and area of each fresh leaf were measured instantly in the field. Leaf thickness was measured using a digital vernier caliper, making sure to avoid the leaf midribs. Leaf area was measured by drawing a leaf outline on A4-sized paper and measuring the area of leaves by the grid method (Radzali et al. 2016). The sampled leaves were kept in between newspapers using the herbarium press and brought to the laboratory. They were oven dried at 80°C for 72 hours and weighed using a digital balance (0.001 g) to determine dry biomass (Pérez-Harguindeguy et al. 2016). Leaf traits and stomatal features (see below) were measured in laboratory during September to November 2020.

Stomatal features

Five leaves, one from each of the previously selected plants, were collected from each quadrat. Stomatal characteristics were determined from surface imprints of the mid-blade abaxial leaf surface (avoiding the leaf margin and main vein) made with clear enamel nail polish (Wang et al. 2014). We randomly selected five stomata in each leaf and measured their length and width under a light microscope with the help of a software coslab (scope image 9.0). For the SD (stomata/mm²), we counted the number of stomata per unit area (mm²) within the images captured at a magnification of 400×. The stomatal apparatus area (As) was calculated following Cai et al. (2014):

$$As = \pi \frac{1}{4} l w$$

where, *l* and *w* denote the length and width of the stomatal apparatus, respectively.

Numerical analysis

Field data were used to calculate density (plants/ha), frequency (%), and basal area (m²/ha) following Zobel et al. (1987). The basal diameter was used to calculate the basal area of each individual rooted in a plot, which was summed to obtain the plot-level basal area of each species. In each elevation band, relative density (RD) of a species was calculated as the ratio of the density of the given species (*d*) to the sum of densities of all species (*D*) and expressed as percentage $RD = (d / D) 100$. In the same way, relative frequency (RF), and relative basal area (RBA) were calculated. The relative values of each species were summed up to obtain the importance value index (IVI) ($IVI = RD + RF + RBA$; Zobel et al. 1987). Following the same method, we calculated the IVI of each species separately in each elevation band. Accordingly, sum of the IVI

of all species at each elevation would be 300. As the basal diameter of trees were ≥ 4 cm, the trees were grouped into five diameter classes starting from 4 cm (4–10, 10–16, 16–22, 22–28, and ≥ 28 cm) to examine the density-diameter relationship following Sharma et al. (2020). The densities of each diameter class were also calculated. Crown cover of each plot was measured by crown densiometer and it was designated in to one of the following four categories: open (crown cover ranging from 0% to 10%), sparse (crown cover ranging from 10% to 30%), moderate (crown cover ranging from 30% to 70%), and closed (crown cover exceeding 70%).

The SLA was determined as the ratio of fresh leaf area to corresponding dry biomass (Pérez-Harguindeguy et al. 2016). Similarly, leaf density (LD) was calculated as the ratio between leaf dry biomass and volume (the product of leaf area and leaf thickness).

To assess the difference in leaf traits (e.g., leaf length, width, thickness, leaf area, leaf dry mass, SLA, LD, SD, stomata length, and stomatal apparatus area) of *R. campanulatum* across different elevations, a one-way analysis of variance (ANOVA) was performed. Once the variables were equal, the least significant difference test was used for multiple comparisons. Pearson's correlation test ($p < 0.05$) was performed to find out the relationship between regeneration (sum of seedling and sapling density), elevation, and crown cover. Moreover, regression analysis between regeneration and tree basal area was also performed to find out their relationship. The relationship of sapling and seedling density with total basal area was assessed using regression analysis. Quadratic regression models showed better fit compared to linear models and higher order polynomials. We selected the quadratic model based on r^2 and p -values (Zar 1999). Before conducting the statistical analyses, the data were checked for normality using the Kolmogorov–Smirnov test and assessed for homogeneity of variance using Levene's test. All the statistical analyses were performed using the Statistical Package for Social Sciences version 24.0 (IBM Co., Armonk, NY, USA).

Results

Community structure

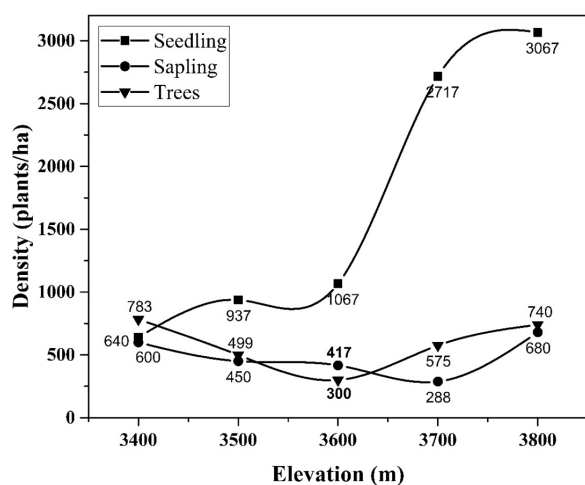
Four tree species, *R. campanulatum*, *B. utilis*, *A. spectabilis*, and *Q. semecarpifolia*, were recorded in the study area (Table 1). The change in tree density revealed a consistent trend across the elevation gradient. It was found that total tree density decreased as elevation increased; it was the highest at 3,400 m a.s.l. and the lowest at 3,800 m a.s.l. (Table 1). The mean basal area of trees was 29.9 ± 7.14 m²ha⁻¹. The variation of basal area followed a unimodal pattern, with the peak occurring at 3,600 m a.s.l. (49 m²/ha) (Table 1).

The IVI of the tree species varied across the elevation gradient. For example, *A. spectabilis* had the highest IVI at

Table 1 Frequency, density, basal area, and importance value index of *Rhododendron campanulatum* and associated tree species along the elevation gradient in the Khali forest, Jumla

Elevation (m a.s.l.)	Species	Frequency (%)	Density (plants/ha)	Basal area (m ² /ha)	IVI
3,400	<i>Abies spectabilis</i>	67	233	19.11	108
	<i>Betula utilis</i>	33	67	6.20	40
	<i>Quercus semecarpifolia</i>	50	233	5.20	58
	<i>Rhododendron campanulatum</i>	50	783	3.17	94
	Total		1,316	33.68	300
3,500	<i>Abies spectabilis</i>	67	165	13.30	74
	<i>Betula utilis</i>	67	182	18.05	88
	<i>Quercus semecarpifolia</i>	67	199	4.68	54
	<i>Rhododendron campanulatum</i>	83	499	2.55	84
	Total		1,045	38.58	300
3,600	<i>Abies spectabilis</i>	50	167	5.05	53
	<i>Betula utilis</i>	100	483	43.14	189
	<i>Rhododendron campanulatum</i>	50	300	0.83	58
	Total		950	49.02	300
3,700	<i>Abies spectabilis</i>	87	50	1.48	56
	<i>Betula utilis</i>	87	300	15.21	152
	<i>Rhododendron campanulatum</i>	25	575	3.40	92
	Total		925	20.09	300
3,800	<i>Betula utilis</i>	80	100	5.53	146
	<i>Rhododendron campanulatum</i>	40	740	2.72	154
	Total		840	8.25	300

IVI: importance value index.

**Fig. 2** Variation of seedling, sapling, and tree density of *Rhododendron campanulatum* with elevation in the Khali forest, Jumla.

3,400 m a.s.l. but at higher elevations it had a relatively low IVI (Table 1). Similarly, *R. campanulatum* was the dominant species with the highest IVI at 3,800 m a.s.l., which corresponds to the tree line. Moving to the lower elevation, *B. utilis* was the dominant species at 3,500–3,700 m a.s.l. while *R. campanulatum* and other species were found as associated tree species.

Regeneration of *Rhododendron campanulatum*

The number of seedlings, saplings, and trees of *R. campanulatum* varied across different elevations. The seedling density increased with increasing elevation, and it was the

Table 2 Correlation coefficients among elevation, crown cover, and regeneration (sum of seedling and sapling density) of *Rhododendron campanulatum*

	Elevation	Crown cover	Regeneration
Crown cover	−0.94**	1	
Regeneration	0.93**	−0.73**	1

** $p < 0.01$.

highest at 3,800 m a.s.l. (Fig. 2). Sapling and tree density also varied with elevation, but without a consistent pattern. The density of *R. campanulatum* saplings decreased from 3,400 to 3,700 m a.s.l. but increased at 3,800 m a.s.l., where it reached its highest density (Fig. 2). Similarly, the tree density of *R. campanulatum* decreased first up to 3,600 m a.s.l. but increased from 3,600 to 3,800 m a.s.l. and the highest tree density was observed at 3,400 m a.s.l. (Fig. 2). Except at one elevation band (3,600 m a.s.l.), sapling density was lower than tree density.

It was observed that open-canopy stands had a greater abundance of *R. campanulatum* seedlings and saplings. The regeneration (seedling + sapling density) showed a negative correlation with tree crown cover (Table 2). The density-diameter curve of *R. campanulatum* in the study area resembled a reverse J-shape, with a decline in the density as tree diameter class increased (Fig. 3). The density of seedling and sapling of *R. campanulatum* is affected by the total basal area ($p < 0.05$). The regression drawn between seedling, and sapling density and elevation showed a significant quadratic relation (Fig. 4).

Leaf traits

Morphological traits

Leaf morphological traits such as, leaf length, width, thickness, leaf area, leaf dry mass, SLA, and LD were significantly affected by elevation (Fig. 5). Specifically, as the elevation raised, leaf length, width, leaf area, and SLA decreased. However, leaf thickness, leaf dry mass, and LD increased with raising elevation (Fig. 5).

Stomatal features

The result of the one-way ANOVA showed that the leaf stomatal traits of *R. campanulatum*, such as SD, stomata length, and stomatal apparatus area, were significantly affected by elevation (Fig. 6). The SD increased with rising elevation, but the stomata length and stomatal apparatus area did not change significantly at lower elevation, but both of them declined significantly at higher elevations.

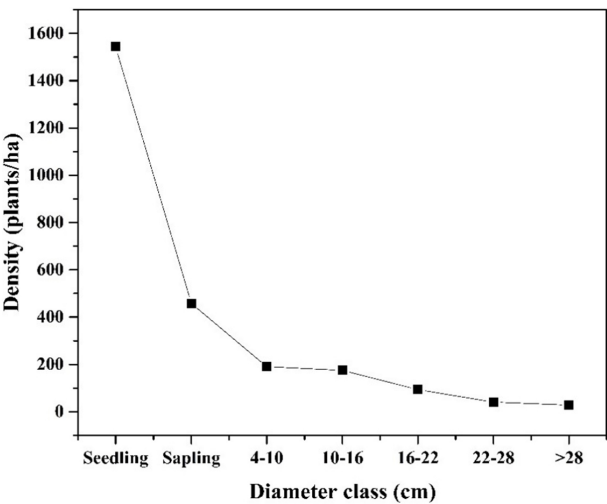


Fig. 3 Density-diameter curve of *Rhododendron campanulatum* in the Khali forest, Jumla.

Discussion

Community structure

Three tree species, namely *A. spectabilis*, *B. utilis*, and *Q. semecarpifolia* were found to be co-occurring with *R. campanulatum* in the study area (Table 1). In fact, *A. spectabilis* and *B. utilis* are the most dominant tall treeline species throughout the Nepal Himalaya, while *R. campanulatum* is the common understory tree species co-occurring with them (Gaire et al. 2014; Liang et al. 2014; Mainali et al. 2020). In addition, we also found *R. campanulatum* forming a pure stand beyond the upper limit of *A. spectabilis*, as observed by Mainali et al. (2020) in Langtang National Park, Central Himalaya.

Regeneration of *Rhododendron campanulatum*

It is well recognized that anthropogenic disturbance and the impacts of global climate change have modified the structure and function of the treeline ecotone (Körner 2012). Stressful environmental conditions naturally prevalent at treeline ecotones cause tree species to struggle for their growth, regeneration, and existence (Rai et al. 2012). However, responses to anthropogenic disturbances and natural stressors are highly species-specific. Therefore, any change in the environmental condition can significantly affect the regeneration of one or another species in the alpine ecotone. In the Western Himalaya, limited regeneration of *R. campanulatum* has been observed along the treeline ecotones (Rai et al. 2012). However, our result revealed that *R. campanulatum* had enhanced regeneration at treeline ecotone (3,800 m) than at lower elevation (3,400 m). Similar findings have been reported for the same species at other study sites in Nepal (e.g., Manaslu Conservation Area [Rana et al. 2016], Langtang National Park [Mainali et al. 2020], Annapurna Conservation Area [Sharma et al. 2020]) and India (e.g., Kedarnath Wild Life Sanctuary of Garhwal/Uttarakhand [Jamloki et al. 2023]). The

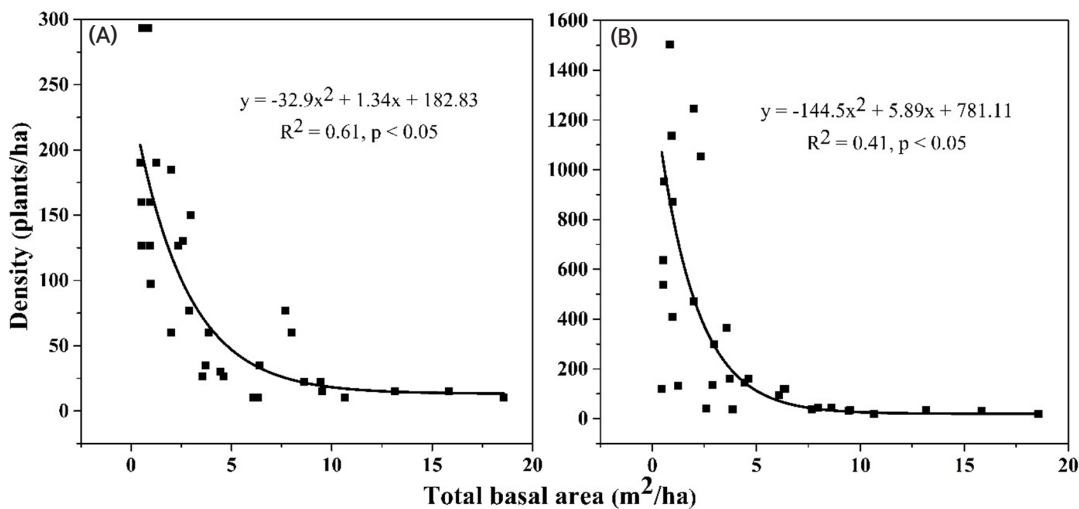


Fig. 4 Relationship of sapling (A) and seedling (B) density (plant/ha) with total basal area (m²/ha).

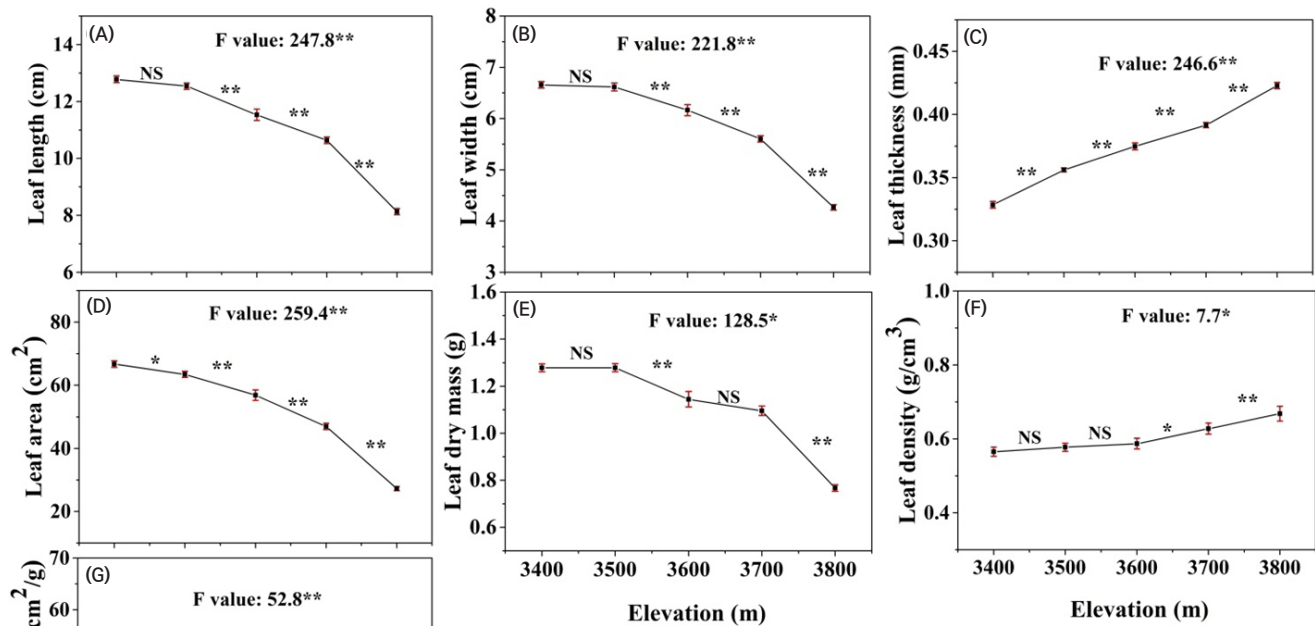


Fig. 5 Variation of leaf morphological traits such as (A) leaf length, (B) leaf width, (C) leaf thickness, (D) leaf area, (E) leaf dry mass, (F) leaf density, (G) specific leaf area of *Rhododendron campanulatum* with elevation in Khali forest, Jumla. NS: no significant difference. * $p < 0.05$, ** $p < 0.01$; ANOVA.

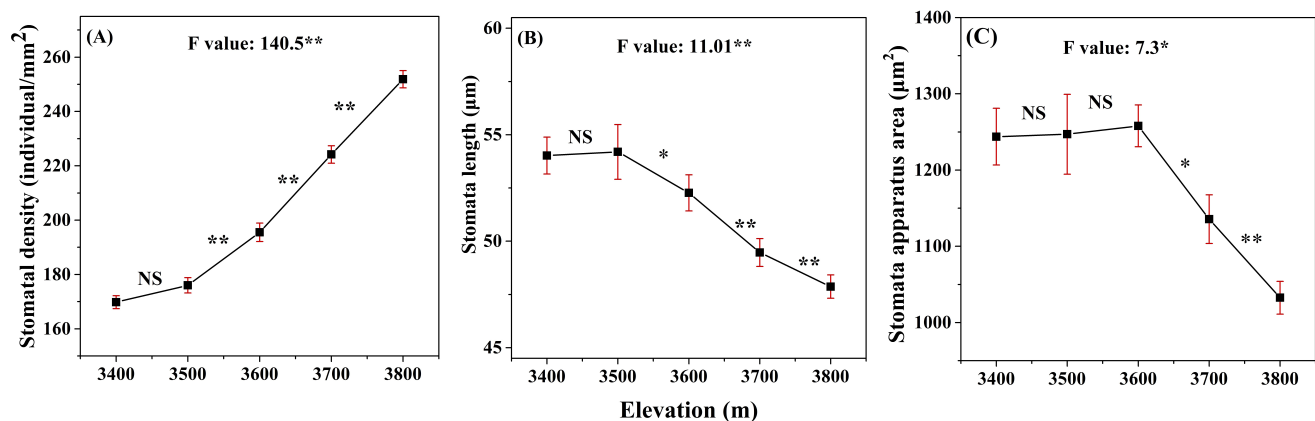


Fig. 6 (A) Variation of leaf stomatal density, (B) stomata length, (C) stomata apparatus area of *Rhododendron campanulatum* with elevation in Khali forest, Jumla. NS: no significant difference. * $p < 0.05$, ** $p < 0.01$; ANOVA.

favorable climatic conditions for seedling establishments, such as direct sunlight, a warmer temperature at microhabitat, and low competition at a higher elevation, might have contributed to the enhanced regeneration of *R. campanulatum* at treeline ecotone. This suggests that *R. campanulatum* has the potential to spread further into alpine grassland in response to climate change. The possibility of *R. campanulatum* expansion to the alpine grasslands of the Nepal Himalaya has also been indicated by previous studies (e.g., Mainali et al. 2020; Sharma et al. 2020). The warming temperatures may facilitate woody vegetation encroachment on alpine habitats by filling open microhabi-

tats and advancing treeline species (Myers-Smith et al. 2011). Furthermore, the presence of the dwarf shrub *Rhododendron anthopogon* above the treeline may provide a warm microhabitat for seedling recruitment and influence the regeneration of *R. campanulatum* at higher elevations (Cavieres and Badano 2009). This facilitative activity of *R. anthopogon* might be the reason why *R. campanulatum* seedlings and saplings were found more frequently within *R. anthopogon* patches compared to open locations observed during sampling (personal observations of DK).

Previous research has described a decrease in crown area with an increase in diameter at breast height and basal area

(Coombes et al. 2019; Mitchell and Popvich 1997). Competition can affect the rate of increase in crown size; canopies can expand when there are fewer neighbours to restrict growth (Verma et al. 2014). Additionally, canopy gaps resulting from environmental conditions provide an opportunity for seedling establishment, increased regeneration, and species richness, while a closed canopy minimizes seedling establishment and regeneration in mountain forests (Dampney et al. 2023; Shrestha et al. 2007). Similarly, a negative relation was observed between the total basal area and seedling and sapling density of *R. campanulatum* which was mainly due to competition for light. Similar results were reported for *Picea engelmannii* in subalpine forest of San Juan Mountains, Colorado (Carlson et al. 2020). A reduction in total basal area at higher elevations contributes to plant density and species richness (Rawat et al. 2018). In this study site, it was observed that the density of *R. campanulatum* was higher in sites with open canopies compared to sites with closed canopies. It is obvious that, as an early successional plant, *R. campanulatum* requires more sunlight for seedling establishment (Mainali et al. 2020). The density distribution of *R. campanulatum* across diameter classes exhibited a non-uniform pattern in the study area. A consistent decline in adult plant density was observed along the elevation gradient, which aligns with the findings of Jamloki et al. (2023) in the Western Himalaya. As the diameter size classes increased, the number of individuals declined, indicating a density-diameter curve that resembles a reverse J-shape. This pattern is typically associated with a stable population structure and continuous regeneration (Malik and Bhatt 2016; Shrestha et al. 2007).

Leaf traits

The leaf characteristics of *R. campanulatum* along an elevational gradient up to the treeline ecotone demonstrated the plant's ability to adapt to stressful environments. Leaves can change their morphological and anatomical features in response to changing habitat conditions since they are directly exposed to the environment (Margaris and Mooney 2012). Additionally, leaf size reflects the functions of the leaf, and it is an important trait of plants to adapt to their environment. In the present study, leaf size (measured as leaf length and width) of *R. campanulatum* decreased with rising elevation; in other words, plants growing at higher elevations have smaller leaves than those at lower elevations. A similar response of leaf size has also been reported in other studies (Liu et al. 2020; Wang et al. 2016; Zhang et al. 2014). The small leaf sizes can reduce the rate of evapotranspiration and the absorption of solar energy, thus mitigating the potential damage caused by intense ultraviolet radiation and strong winds prevalent at high elevations (Tian et al. 2016). Accordingly, smaller leaves of *R. campanulatum* at higher elevations can be a significant adaptation of this species to withstand cold

temperatures, strong solar radiation, and wind exposure.

The SLA has been widely used in plant functional ecology, agriculture, and forestry to understand carbon gain from individual leaf to entire canopy (Poorter et al. 2009). It is a key feature in plant growth that is closely related to photosynthesis and relative growth rate (Cornelissen et al. 2003; Shi et al. 2020). In general, higher SLA is linked to greater photosynthetic efficiency (Zhang et al. 2020) while the lower SLA observed a phenotypical adaptations of plants to harsh environments (Halbritter et al. 2018). To improve mechanical strength and reduce water loss, the SLA frequently decreases in cold temperatures and/or windy conditions (Kudo et al. 1999). Accordingly, the lower SLA of *R. campanulatum* at higher elevation may enable plants to withstand low temperatures and windy conditions. A similar decline in SLA with rising elevation has been reported previously in *R. lepidotum* (Pandey et al. 2021), *R. anthopogon* (Rathore et al. 2018), *R. capitatum* (Yang et al. 2022), and *R. campanulatum* (Jamloki et al. 2023; Sharma et al. 2020).

For a better understanding of how plant species adapt or react to shifting environmental conditions over large geographical scales, SD and their size are preferable traits (Hetherington and Woodward 2003; Woodward 1986). Stomata serve as essential conduits for the exchange of CO₂ and H₂O between the interior leaf space and the outside environment (Wang et al. 2014). Therefore, SD is a crucial characteristic that controls this exchange. An increase in SD with rising elevation that we observed in *R. campanulatum* is similar to the findings of previous studies by Wang et al. (2014) and Yang et al. (2014) in Tibetan and Mongolian grassland species. According to Pato and Obeso (2012), the CO₂ availability theory may help to explain the SD and elevation relationship. The theory suggests that SD is influenced by the partial pressure of CO₂. Plants may increase SD at higher elevations to enhance gas absorption due to the lower CO₂ and O₂ levels. Higher stomatal densities facilitate efficient CO₂ diffusion for photosynthesis, which promotes plant survival in high-elevation environments (Medlyn et al. 2011; Royer 2001). Increased SD in response to low CO₂ is a functional adaptation in high-elevation environments (Kelly and Beerling 1995).

Conclusions

Gaining knowledge about the regeneration potential and leaf traits of *R. campanulatum* in the treeline ecotone is crucial for understanding the impact of climate change on the position and functioning of treeline ecosystems. The remarkable regeneration of *R. campanulatum* at higher elevations serves as evidence of its successful adaptation to the environmentally stressful conditions. Canopy gaps may provide an opportunity for seedling establishment and

higher regeneration. The successful regeneration of *R. campanulatum* in such adverse environmental conditions may lead to the expansion of this species to higher elevations in the studied region. Additionally, lower SLA with the high SD in the leaves at the upper limit of the species distribution suggested that *R. campanulatum* is well adapted at its upper distribution range. However, long-term monitoring is necessary to better understand its regeneration dynamics and fitness above the treeline.

Abbreviations

SLA: Specific leaf area

SD: Stomatal density

RD: Relative density

RF: Relative frequency

RBA: Relative basal area

IVI: Importance value index

LD: Leaf density

Acknowledgements

We express our gratitude to Division Forest Office Jumla.

Authors' contributions

BBS and DK conceptualized the research. DK, BP, RB, and DB conducted field work. DK, BG, and SB conducted the experimental work at laboratory and analyzed the data. DK drafted the first copy of the manuscript. BBS and BG critically commented and revised the manuscript. All the authors read and approved the final version of the manuscript.

Funding

Not applicable.

Availability of data and materials

The data that supports the findings of this study will be made available on request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

- Acharya KP, Paudel PK. Biodiversity in Karnali province: current status and conservation. Surkhet: Ministry of Industry, Tourism, Forest and Environment, Karnali Province Government; 2020.
- Bisht VK, Kuniyal CP, Bhandari AK, Nautiyal BP, Prasad P. Phenology of plants in relation to ambient environment in a subalpine forest of Uttarakhand, western Himalaya. *Physiol Mol Biol Plants*. 2014; 20(3):399–403. <https://doi.org/10.1007/s12298-014-0238-2>.
- Cai YF, Li SF, Li SF, Xie WJ, Song J. How do leaf anatomies and photosynthesis of three *Rhododendron* species relate to their natural environments? *Bot Stud*. 2014;55(1):36. <https://doi.org/10.1186/1999-3110-55-36>.
- Carlson AR, Sibold JS, Negrón JF. Canopy structure and below-canopy temperatures interact to shape seedling response to disturbance in a Rocky Mountain subalpine forest. *For Ecol Manag*. 2020;472:118234. <https://doi.org/10.1016/j.foreco.2020.118234>.
- Cavieres LA, Badano EI. Do facilitative interactions increase species richness at the entire community level? *J Ecol*. 2009;97(6):1181–91. <https://doi.org/10.1111/j.1365-2745.2009.01579.x>.
- Chakraborty A, Saha S, Sachdeva K, Joshi PK. Vulnerability of forests in the Himalayan region to climate change impacts and anthropogenic disturbances: a systematic review. *Reg Environ Chang*. 2018;18: 1783–99. <https://doi.org/10.1007/s10113-018-1309-7>.
- Chen G, Yang K, Zhang J, Wang M, Wang L, Xian J. Effects of succession stages and altitudinal gradient on leaf surface area and biomass allocation of typical plants in the subalpine of Eastern Tibetan Plateau. *Glob Ecol Conserv*. 2021;27:e01590. <https://doi.org/10.1016/j.gecco.2021.e01590>.
- Coomes A, Martin J, Slater D. Defining the allometry of stem and crown diameter of urban trees. *Urban For Urban Green*. 2019;44: 126421. <https://doi.org/10.1016/j.ufug.2019.126421>.
- Cornelissen JHC, Lavorel S, Garnier E, Díaz S, Buchmann N, Gurvich DE, et al. A handbook of protocols for standardised and easy measurement of plant functional traits worldwide. *J Aust J Bot*. 2003; 51(4):335–80. <https://doi.org/10.1071/BT02124>.
- Damptey FG, Adofo E, Duah-Gyamfi A, Adusu D, Opuni-Frimpong E. Logging effects on seedling regeneration and diversity in a tropical moist semi-deciduous forest in Ghana. *Geol Ecol Landsc*. 2023;7(4): 269–80. <https://doi.org/10.1080/24749508.2021.1952769>.
- De Deus Vidal J, le Roux PC, Johnson SD, te Beest M, Clark VR. Beyond the tree-line: the C3-C4 “grass-line” can track global change in the world’s grassy mountain systems. *Front Ecol Evol*. 2021;9: 760118. <https://doi.org/10.3389/fevo.2021.760118>.
- Department of Forest and Soil Conservation (DoFSC). *Rhododendron Conservation Strategy (2075–2080)*. Kathmandu: Department of Forest and Soil Conservation, Ministry of Forest and Environment; 2019.
- Department of Hydrology and Meteorology (DHM). *Observed climate trend analysis in the districts and physiographic regions of Nepal (1971–2014)*. Kathmandu: Department of Hydrology and Meteorology; 2017.
- Gaire NP, Koirala M, Bhuju DR, Borgaonkar HP. Treeline dynamics with climate change at the central Nepal Himalaya. *Clim Past*. 2014; 10(4):1277–90. <https://doi.org/10.5194/cp-10-1277-2014>.
- Gaire NP, Koirala M, Bhuju DR, Carrer M. Site- and species-specific treeline responses to climatic variability in eastern Nepal Himalaya. *Dendrochronologia*. 2017;41:44–56. <https://doi.org/10.1016/j.dendro.2016.03.001>.
- Grabherr G, Gottfried M, Paull H. Climate effects on mountain plants. *Nature*. 1994;369(6480):448. <https://doi.org/10.1038/369448a0>.

- Grace J, Berninger F, Nagy L. Impacts of climate change on the tree line. *Ann Bot.* 2002;90(4):537-44. <https://doi.org/10.1093/aob/mcf222>.
- Halbritter AH, Fior S, Keller I, Billeter R, Edwards PJ, Holderegger R, et al. Trait differentiation and adaptation of plants along elevation gradients. *J Evol Biol.* 2018;31(6):784-800. <https://doi.org/10.1111/jeb.13262>.
- Hetherington AM, Woodward FI. The role of stomata in sensing and driving environmental change. *Nature.* 2003;424(6951):901-8. <https://doi.org/10.1038/nature01843>.
- Huang W, Reddy GVP, Li Y, Larsen JB, Shi P. Increase in absolute leaf water content tends to keep pace with that of leaf dry mass- evidence from bamboo plants. *Symmetry.* 2020;12(8):1345. <https://doi.org/10.3390/sym12081345>.
- Jamloki A, Singh A, Chandra S, Shukla V, Nautiyal MC, Malik ZA. Population structure, regeneration potential and leaf morphological traits of *Rhododendron campanulatum* D.Don along an altitudinal gradient in Western Himalaya. *Plant Biosyst.* 2023;157(1):159-74. <https://doi.org/10.1080/11263504.2022.2098866>.
- Kelly CK, Beerling DJ. Plant life form, stomatal density and taxonomic relatedness: a reanalysis of Salisbury (1927). *Funct Ecol.* 1995;9(3):422-31. <https://doi.org/10.2307/2390005>.
- Körner C. Alpine treelines: functional ecology of the global high elevation tree limits. Basel: Springer; 2012.
- Kudo G, Nordenhäll U, Molau U. Effects of snowmelt timing on leaf traits, leaf production, and shoot growth of alpine plants: comparisons along a snowmelt gradient in northern Sweden. *Écoscience.* 1999;6(3):439-50. <https://doi.org/10.1080/11956860.1999.11682543>.
- Liang E, Dawadi B, Pederson N, Eckstein D. Is the growth of birch at the upper timberline in the Himalayas limited by moisture or by temperature? *Ecology.* 2014;95(9):2453-65. <https://doi.org/10.1890/13-1904.1>.
- Liu W, Zheng L, Qi D. Variation in leaf traits at different altitudes reflects the adaptive strategy of plants to environmental changes. *Ecol Evol.* 2020;10(15):8166-75. <https://doi.org/10.1002/ece3.6519>.
- Lütz C. Cell physiology of plants growing in cold environments. *Protoplasma.* 2010;244(1-4):53-73. <https://doi.org/10.1007/s00709-010-0161-5>.
- Mainali K, Shrestha BB, Sharma RK, Adhikari A, Gurarie E, Singer M, et al. Contrasting responses to climate change at Himalayan treelines revealed by population demographics of two dominant species. *Ecol Evol.* 2020;10(3):1209-22. <https://doi.org/10.1002/ece3.5968>.
- Malik ZA, Bhatt AB. Regeneration status of tree species and survival of their seedlings in Kedarnath Wildlife Sanctuary and its adjoining areas in Western Himalaya, India. *Trop Ecol.* 2016;57(4):677-90.
- Margaris NS, Mooney HA. Components of productivity of Mediterranean-climate regions Basic and applied aspects: proceedings of the International symposium on photosynthesis, primary production and biomass utilization in Mediterranean-type ecosystems, held in Kassandra, Greece, September 13-15, 1980. Dordrecht: Springer; 2012.
- Medlyn BE, Duursma RA, Eamus D, Ellsworth DS, Prentice IC, Barton CVM, et al. Reconciling the optimal and empirical approaches to modelling stomatal conductance. *Glob Chang Biol.* 2011;17(6):2134-44. <https://doi.org/10.1111/j.1365-2486.2010.02375.x>.
- Mishra AK, Behera SK, Singh K, Mishra RM, Chaudhary LB, Singh B. Effect of abiotic factors on understory community structures in moist deciduous forests of northern India. *For Sci Pract.* 2013;15:261-73. <https://doi.org/10.1007/s11632-013-0415-3>.
- Mitchell JE, Popovich SJ. Effectiveness of basal area for estimating canopy cover of ponderosa pine. *For Ecol Manag.* 1997;95(1):45-51. [https://doi.org/10.1016/S0378-1127\(97\)00002-9](https://doi.org/10.1016/S0378-1127(97)00002-9).
- Myers-Smith IH, Forbes BC, Wilmking M, Hallinger M, Lantz T, Blok D, et al. Shrub expansion in tundra ecosystems: dynamics, impacts and research priorities. *Environ Res Lett.* 2011;6(4):045509. <https://doi.org/10.1088/1748-9326/6/4/045509>.
- Pandey M, Pathak ML, Shrestha BB. Morphological and wood anatomical traits of *Rhododendron lepidotum* Wall ex G. Don along the elevation gradients in Nepal Himalayas. *Arct Antarct Alp Res.* 2021;53(1):35-47. <https://doi.org/10.1080/15230430.2020.1859719>.
- Pato J, Obeso JR. Growth and reproductive performance in bilberry (*Vaccinium myrtillus*) along an elevation gradient. *Écoscience.* 2012;19(1):59-68. <https://doi.org/10.2980/19-1-3407>.
- Pauli H, Gottfried M, Lamprecht A, Niessner S, Rumpf S, Winkler M, et al. The GLORIA field manual - standard Multi-Summit approach, supplementary methods and extra approaches. 5th ed. Vienna: GLORIA-Coordination, Austrian Academy of Sciences & University of Natural Resources and Life Sciences; 2015.
- Pérez-Harguindeguy N, Díaz S, Garnier E, Lavorel S, Poorter H, Jaureguiberry P, et al. *Corrigendum to: new handbook for standardised measurement of plant functional traits worldwide.* *J Aust J Bot.* 2016;64(8):715-6. https://doi.org/10.1071/BT12225_CO.
- Pokhriyal P, Uniyal P, Chauhan DS, Todaria NP. Regeneration status of tree species in forest of Phakot and Pathri Rao watersheds in Garhwal Himalaya. *Curr Sci.* 2010;98(2):171-5.
- Polunin O, Stainton A. Concise flowers of the Himalaya. Delhi: Oxford University Press; 1987.
- Poorter H, Niinemets Ü, Poorter L, Wright IJ, Villar R. Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytol.* 2009;182(3):565-88. <https://doi.org/10.1111/j.1469-8137.2009.02830.x>. Erratum in: *New Phytol.* 2009;183(4):1222.
- Radzali MH, Kamal NAM, Diah NM. Measuring leaf area using Otsu segmentation method (LAMOS). *Indian J Sci Technol.* 2016;9(48):1-6. <https://doi.org/10.17485/ijst/2016/v9i48/109307>.
- Rai ID, Adhikari BS, Rawat GS, Bargal K. Community structure along timberline ecotone in relation to micro-topography and disturbances in Western Himalaya. *Not Sci Biol.* 2012;4(2):41-52. <https://doi.org/10.15835/nsb427411>.
- Rana P, Koirala M, Bhuju DR, Boonchird C. Population structure of *Rhododendron campanulatum* D. Don and associated tree species along the elevational gradient of Manaslu conservation area, Nepal. *J Inst Sci Technol.* 2016;21(1):95-102. <https://doi.org/10.3126/jist.v21i1.16058>.
- Rathore N, Thakur D, Chawla A. Seasonal variations coupled with elevation gradient drives significant changes in eco-physiological and biogeochemical traits of a high altitude evergreen broadleaf shrub, *Rhododendron anthopogon*. *Plant Physiol Biochem.* 2018;132:708-19. <https://doi.org/10.1016/j.plaphy.2018.08.009>.
- Rawat DS, Dash SS, Sinha BK, Kumar V, Banerjee A, Singh P. Community structure and regeneration status of tree species in Eastern Himalaya: a case study from Neora Valley National Park, West Bengal,

- India. Taiwan. 2018;63(1):16-24. <https://doi.org/10.6165/tai.2018.63.16>.
- Royer DL. Stomatal density and stomatal index as indicators of paleoatmospheric CO₂ concentration. *Rev Palaeobot Palynol*. 2001;114(1-2):1-28. [https://doi.org/10.1016/s0034-6667\(00\)00074-9](https://doi.org/10.1016/s0034-6667(00)00074-9).
- Schwab N, Schickhoff U, Bürzle B, Müller M, Böhner J, Chaudhary RP, et al. Implications of tree species – environment relationships for the responsiveness of Himalayan krummholz treelines to climate change. *J Mt Sci*. 2017;14:453-73. <https://doi.org/10.1007/s11629-016-4257-z>.
- Sharma KP, Tiwari A, Shrestha BB. Changes in regeneration and leaf traits of *Rhododendron campanulatum* along a treeline ecotone in central Nepal. *J Mt Sci*. 2020;17(3):602-13. <https://doi.org/10.1007/s11629-019-5386-y>.
- Shi P, Niinemets Ü, Hui C, Niklas KJ, Yu X, Hölscher D. Leaf bilateral symmetry and the scaling of the perimeter vs. the surface area in 15 vine species. *Forests*. 2020;11(2):246. <https://doi.org/10.3390/f11020246>.
- Shrestha BB, Ghimire B, Lekhak HD, Jha PK. Regeneration of treeline birch (*Betula utilis* D. Don) forest in a trans-himalayan dry valley in central Nepal. *Mt Res Dev*. 2007;27(3):259-67. <https://doi.org/10.1659/mrdd.0784>.
- Singh P, Arya V, Negi GCS, Singh SP. Expansion of *Rhododendron campanulatum* krummholz in the treeline ecotone in Tungnath, Garhwal Himalaya. *Trop Ecol*. 2018;59(2):287-95.
- Singh SP, Sharma S, Dhyani PP. Himalayan arc and treeline: distribution, climate change responses and ecosystem properties. *Biodivers Conserv*. 2019;28(8):1997-2016. <https://doi.org/10.1007/s10531-019-01777-w>.
- Taylor SH, Franks PJ, Hulme SP, Spriggs E, Christin PA, Edwards EJ, et al. Photosynthetic pathway and ecological adaptation explain stomatal trait diversity amongst grasses. *New Phytol*. 2012;193(2):387-96. <https://doi.org/10.1111/j.1469-8137.2011.03935.x>.
- Tian M, Yu G, He N, Hou J. Leaf morphological and anatomical traits from tropical to temperate coniferous forests: mechanisms and influencing factors. *Sci Rep*. 2016;6:19703. <https://doi.org/10.1038/srep19703>.
- Tiwari A, Fan ZX, Jump AS, Li SF, Zhou ZK. Gradual expansion of moisture sensitive *Abies spectabilis* forest in the Trans-Himalayan zone of central Nepal associated with climate change. *Dendrochronologia*. 2017;41:34-43. <https://doi.org/10.1016/j.dendro.2016.01.006>.
- Tiwari A, Jha PK. An overview of treeline response to environmental changes in Nepal Himalaya. *Trop Ecol*. 2018;59(2):273-85.
- Tiwari OP, Rana YS, Krishan R, Sharma CM, Bhandari BS. Regeneration dynamics, population structure, and forest composition in some ridge forests of the Western Himalaya, India. *For Sci Technol*. 2018;14(2):66-75. <https://doi.org/10.1080/21580103.2018.1447517>.
- Vandvik V, Halbritter AH, Yang Y, He H, Zhang L, Brummer AB, et al. Plant traits and vegetation data from climate warming experiments along an 1100m elevation gradient in Gongga Mountains, China. *Sci Data*. 2020;7(1):189. <https://doi.org/10.1038/s41597-020-0529-0>.
- Verma NK, Lamb DW, Reid N, Wilson B. An allometric model for estimating DBH of isolated and clustered Eucalyptus trees from measurements of crown projection area. *For Ecol Manag*. 2014;326:125-32. <https://doi.org/10.1016/j.foreco.2014.04.003>.
- Wang R, Yu G, He N, Wang Q, Xia F, Zhao N, et al. Elevation-related variation in leaf stomatal traits as a function of plant functional type: evidence from Changbai Mountain, China. *PLoS One*. 2014;9(12):e115395. <https://doi.org/10.1371/journal.pone.0115395>.
- Wang YY, Qi DH, Liu WS, Liang WB. Comparison on leaf phenotypic and anatomical structures of *Polygonum paleaceum* along altitudinal gradients at Yulong mountains. *Acta Bot Boreal-Occident Sin*. 2016;36(1):70-7.
- Wielgolaski FE, Hofgaard A, Holtmeier FK. Sensitivity to environmental change of the treeline ecotone and its associated biodiversity in European mountains. *Clim Res*. 2017;73(1-2):151-66.
- Woodward FI. Ecophysiological studies on the shrub *Vaccinium myrtillus* L. taken from a wide altitudinal range. *Oecologia*. 1986;70(4):580-6. <https://doi.org/10.1007/BF00379908>.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, et al. The worldwide leaf economics spectrum. *Nature*. 2004;428(6985):821-7. <https://doi.org/10.1038/nature02403>.
- Yang KT, Chen GP, Xian JR, Yu XY, Zhang JW, Wang L. Characteristics of stem and leaf functional traits of *Rhododendron capitatum* on the north slope of Zhagaliang, Gansu. *Acta Prataculturae Sin*. 2022;31(2):111-20. <https://doi.org/10.11686/cyxb2020536>.
- Yang X, Yang Y, Ji C, Feng T, Shi Y, Lin L, et al. Large-scale patterns of stomatal traits in Tibetan and Mongolian grassland species. *Basic Appl Ecol*. 2014;15(2):122-32. <https://doi.org/10.1016/j.baae.2014.01.003>.
- Zar JH. Biostatistical analysis. 4th ed. Upper Saddle River: Prentice Hall; 1999.
- Zhang CY, Wang Y, Li MS, Xu MZ, Liu XH. Comparative analysis of anatomical structure of *Rhododendron chrysanthum* leaves at different altitudes in Changbai Mountain area. *J Agric Sci Yanbian Univ*. 2014;36:39-43.
- Zhang L, Luo T, Zhu H, Daly C, Deng K. Leaf life span as a simple predictor of evergreen forest zonation in China. *J Biogeogr*. 2010;37(1):27-36. <https://doi.org/10.1111/j.1365-2699.2009.02170.x>.
- Zhang L, Yang L, Shen W. Dramatic altitudinal variations in leaf mass per area of two plant growth forms at extreme heights. *Ecol Indic*. 2020;110:105890. <https://doi.org/10.1016/j.ecolind.2019.105890>.
- Zhang Y, Zheng ZH, Zhang ZX. Community structure and regeneration types of *Betula dahurica* forest in Badaling forest center of Beijing. *For Stud China*. 2007;9:152-6. <https://doi.org/10.1007/s11632-007-0024-0>.
- Zobel DB, Jha PK, Behan MJ, Yadav UKR. A practical manual for ecology. Kathmandu: Ratna Book Distributors; 1987.
- Zobel DB, Singh SP. Himalayan forests and ecological generalizations. *BioScience*. 1997;47(11):735-45. <https://doi.org/10.2307/1313096>.