Pak. J. Bot., 47(6): 2115-2125, 2015.

ENVIRONMENTAL FEEDBACKS OF THE SUBALPINE ECOTONE SPECIES IN THE LANGTANG NATIONAL PARK, CENTRAL NEPAL HIMALAYA

KUBER PRASAD BHATTA[1,*], MAAN BAHADUR ROKAYA[2,3] AND ZUZANA MÜNZBERGOVÁ[2,4]

[1]Department of Geography, University of Bergen, Post Box 7802, 5020 Bergen, Norway
[2]Institute of Botany, Czech Academy of Sciences, Zamek 1, CZ-252 43 Průhonice, Czech Republic
[3]Department of Biodiversity Research, Global Change Research Centre, Czech Academy of Sciences, Bělidla 4a, 603 00 Brno, Czech Republic
[4]Department of Botany, Faculty of Sciences, Charles University, Benátská 2, 128 01 Praha, Czech Republic
**Corresponding author's e-mail: Kuber.Bhatta@geog.uib.no*

Abstract

An acute sensitivity of ecotone vegetation to environmental changes makes ecotone vegetation dynamics as one of the prime aspects in vegetation ecology. Understory vegetation of ecotone can indicate the changing environmental conditions of the region by showing its own growth pattern, abundance, and assemblages. Our study aims at investigating the compositional pattern of herbaceous species of a subalpine ecotone in Langtang National Park, central Nepal and analyzing their responses to the different environmental factors. We performed multivariate analyses to study species composition, and for analyzing individual plant species responses logistic regression was used.

It was observed that profound variation in the canopy cover and soil organic carbon along the ecotone significantly influenced the species composition of the herbaceous vegetation. Response curve analysis showed the most significant response of eleven least tolerance species to the environmental variables. *Gentiana argentea, Geranium donianum, Kobresia* sp., *Potentilla griffithii, Rubia wallichiana, Rubus nepalensis, Thalictrum chelidonii* and *Thalictrum cultratum* were particularly important because these species had narrow amplitude to both the studied environmental variables - canopy cover and soil organic carbon. As these species could be critically sensitive to even minor fluctuation in the environmental factor(s), can be regarded as potential indicator species of the respective environmental variable. These indicator species could be vital in understanding community dynamics of the vulnerable ecotone vegetation within the National Park via their use in long-term assessment of the habitat conditions. Replicated studies at broader scales would further be important for understanding the dynamics of the other ecotone plant communities of Nepal Himalaya.

Key words: Ecological amplitude/niche, Ecotone vegetation, Indicator species, Species response curve, Multivariate analysis, Nepal Himalaya.

Introduction

Plant communities are complex dynamic assemblages of species filtered from the regional pool by environmental factors at different scales. Distribution, abundance, and coexistence of plant species are the results of interacting local climatic, edaphic (Richardson *et al.*, 1995; Pausas & Austin, 2001), and biotic factors (Dunnett & Grime, 1999; Klanderud, 2008). Different species have specific requirements of environmental conditions to grow and survive in the nature. The range of these environmental conditions is termed as 'ecological amplitude or niche' of the species. Any alternation of environmental condition(s) therefore leads to change in abundance of individual plant species (Lindborg *et al.*, 2005) or the total composition of species in an ecosystem (Smart, 2000). In the last few decades, there are reports of alarming signals of global climate and land-use changes across globe, and the impacts of these changes are more visible on the alpine and arctic regions of the world (Hartley *et al.*, 2012; Anon., 2013). These changes have shown profound effects on species interactions or on single species abundances at different scales (Klanderud, 2008). As various species might respond differently to environmental changes, creation of a new plant community is more feasible than migration of already existing communities (Gigauri *et al.*, 2013).

Plant communities in the high Himalayan mountains often show sharp compositional changes over space which are known as ecotones (Shrestha & Vetaas, 2009; Khan *et al.*, 2013). Ecotone vegetation dynamics has become a prime issue in community ecology because there is an acute sensitivity of the vegetation to the environmental changes at local as well as global level (Shrestha & Vetaas, 2009; D'Odorico *et al.*, 2013). Most of the ecotone studies focus on tree line ecotones along elevational gradients (e.g. Gottfried *et al.*, 1998; Shrestha & Vetaas, 2009). The changes along these ecotones may not be only due to the presence of the ecotone, but also due to change in other environmental factors changing with elevation (Vetaas & Grytnes, 2002; Khan *et al.*, 2013). Therefore, in addition to the above mentioned studies, it is also important to focus on ecotones at the same elevation (horizontal ecotones) because such ecotones can clearly reveal the effects of local edaphic and climatic factors on species richness and composition of plant species at the same landscape level.

Most of the existing studies use data collected at meso- or macro-scale. (e.g. Eppinga *et al.*, 2013; Lutz *et al.*, 2013) and the studies on species-specific and micro-environmental changes of the horizontal ecotones at a local scale are lacking. Such studies are important as they could lead to detection of indicator species, which could then be used for monitoring of micro-level ecosystem changes. Therefore, the present study is designed to understand individualistic responses of the species to the environmental factors at the critical grassland-shrubland-young forest-old forest ecotone of Langtang National Park, central Nepal.

There are various ways to study species responses to environmental changes. The most common method is to use species composition to predict environmental variables, known as bio-indication (Diekmann, 2003; Peppler-Lisbach, 2008). Doing this, however, requires some preliminary knowledge on species requirements such as Ellenberg indicator values (Ellenberg *et al.*, 1991). Such values permit users to produce statements on the ecology of a site without carrying out instrumental measurements (Persson, 1981; Hill *et al.*, 1999). Unfortunately, such information is not available for most part of the world. The other type of indicator species approaches are based on likelihood method used to create species response curves (optima and tolerance values of the response curves) to predict environmental niche (Gégout *et al.*, 2003; Wamelink *et al.*, 2007). This is based on the fact that each species has its own amplitude with respect to habitat variable(s) (Ellenberg *et al.*, 1991; Naqinezhad *et al.*, 2013). Recently there has also been an increasing trend in use of modelling of the distribution of species and communities, to test biogeographical hypotheses, and to define conservation priorities (Naqinezhad *et al.*, 2013).

The composition of understory vegetation reflects edaphic factors, and therefore could be used to evaluate site conditions, predict forest composition, and classify habitats (Naqinezhad *et al.*, 2013). This is because herbaceous understory vegetation is more sensitive than the canopy forming species to the changes in microenvironmental condition(s). Furthermore, moisture and light regimes differ greatly between canopy forming trees and the understory vegetation. We, therefore, made a case study focusing only on the compositional change of understory herbaceous vegetation and species-environment relationship in the horizontal ecotone of the subalpine zone in Langtang valley, central Nepal. In the present study, we attempted to answer the following questions: (a) What is the pattern of species richness and composition in an ecotone of high altitude vegetation in Langtang valley?, (b) What are the responses of herbaceous species along ecotone to different environmental factors? (c) What are the indicator species, which show significant responses to the environmental changes? In order to answer the above-mentioned questions, we laid horizontal transects in a grassland-shrubland-young forest-old forest ecotone within the Langtang National Park, Nepal. We analyzed the data to find the pattern of species richness and composition along the ecotone and highlighted the indicator species with narrow amplitudes.

Material and Methods

Study area: The field work was carried out during March-April (pre-monsoon season) and July-August (post-monsoon season) 2009 in a grassland-shrubland-young forest-old forest ecotone close to Ghodatabela village (3100-3200m above sea level) in Langtang valley, central Nepal (Fig. 1). The study site is a flat riverbank that extends from northeast to southwest along the Langtang River bank at 28° 11′ N latitudes and 85°27′ E longitudes.

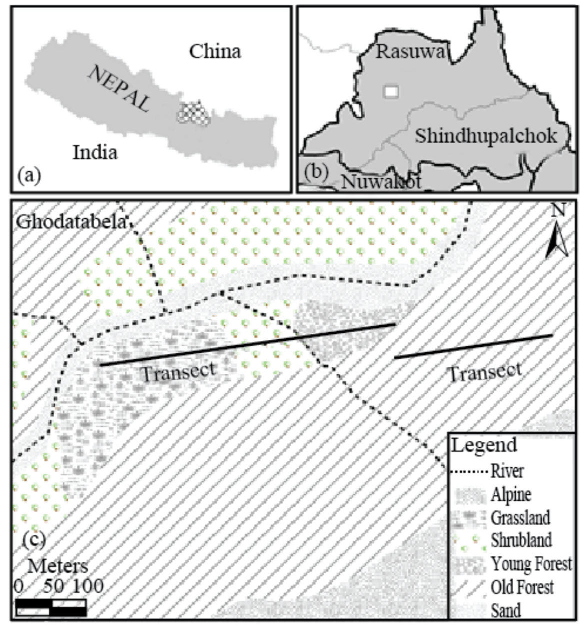


Fig. 1. Location map of the study area; (a) location of the Langtang National Park within the country, (b) sampling site (Small Square) within the National Park, (c) detailed map of the sampling site.

The ecotone has a transition zone that consists of shrubland and young (*Salix-Hippophae*) forest, with *Berberis aristata, Hippophae salicifolia, Piptanthus nepalensis, Rhododendron arboreum*, and *Salix longiflora*. *Tsuga dumosa* is the dominant canopy tree in the old forest. All the four different physiognomic vegetation types are present at about same elevation along our sampling transects. Grassland is located along the banks of Langtang River and was appropriated by local residents as a cultivated land in the past (50-60 years ago). However, since past 40-45 years the land has been used as 'goth or cattle shelter' during seasonal grazing. Soil of the grassland is therefore mixed loam-silt type. The shrubland and young (*Salix-Hippophae*) forest is developed on the glacial moraine. Soil of the shrubland and young forest is calcareous and consists mainly of sand, gravel, and rocks. Litter and organic matter is scanty in the soil. The old forest with dense canopy is rich in humus and litter content. The climate of the region is cold temperate type with annual average rainfall of 652 mm. Snow is common in winter. The mean monthly maximum-minimum temperatures recorded from the nearest Kyanjing station (3600m above sea level) during winter were between 4.7ºC and -4ºC and for summer were 10.5 and 4.5 ºC (unpublished data of Department of Meteorology and Hydrology, Nepal from year 1988-2002).

Transhumance and agriculture are the main land-use practices in the Langtang valley. The semi-natural grasslands are grazed by horses, yaks, and cows during summer. Our sampling site lies in the transition zone between the main villages (in temperate areas) and the higher alpine belt. Therefore the area is occupied twice by cattle in the same year – first during early summer when the cattle are gradually moved up in the alpine pastures for seasonal grazing, and the secondly during mid-winter when the cattle are moved down to villages. The whole area thus is subjected to intense grazing pressure.

Sampling strategy

Vegetation sampling: In order to have full representation of the species as well as environmental heterogeneity we used two parallel transects of about 180m length lying 15m apart in east-west direction. Each transect was divided into three equal segments of about 60m length which were laid on each vegetation types. These transects are stretched from the grassland into the shrubland and young forest. In order to study the old forest, which is located approximately 50m south of the last part of the transect, two separate additional transects of ~ 60m length were laid down. Along the transect, we used ten plots of $1m^2$ (1m x 1m) separated by 5m interval in each of the four vegetation types (grassland, shrubland, young forest, and old forest). Overall, there were 80 sample plots, 20 plots in each vegetation type. Pre-monsoon (March-April) is a drier and warmer season of the year when the cattle herds start shifting to higher elevations after intensive grazing in our study area. Consequently, the study area remains dry and very sparsely covered with the herbs during the period. Most of the herbs germinate and bloom during the monsoon and post monsoon season, after plenty of rainfall during the monsoon season. Therefore, we sampled vegetation only during the post-monsoon season. However, we collected the soil samples from each plot during pre- as well as post-monsoon season and used averaged values of soil parameters. This is because some of the soil properties such as soil moisture and soil pH may vary due to the rainfall during the monsoon season.

We recorded only herbaceous species in each vegetation type so that canopy cover can be treated as an external environmental factor that affects plant distribution at fine scale. Each plot was divided into four equal sub-plots for a careful census of the vegetation. The rooted presence (1) and absence (0) values were designated for herbaceous species in each sub-plot. This gave an abundance scale (0-4) of each species within a plot. However, only the presence (1) or absence (0) of each species in each plot was used for response curve analyses. We also counted number of species per plot (referred to as species richness in subsequent text).

Plant identification and nomenclature: Most of the species were identified in the field during sampling. The unidentified species were collected with proper field notes and brought to the Central Department of Botany, Tribhuvan University, Nepal. They were identified with the help of experts from the department. We followed the nomenclature of Press *et al*. (2000).

Sampling of environmental variables and analysis: Canopy cover is commonly regarded as a surrogate for light and was estimated using normal digital photography technique. A digital camera (Sony Cybershot 3.0.02.12110) was pointed towards the sky and snap-shots taken from the center of each plot and whole space above the plot covered in each photograph. The screen of the camera was scaled with grid lines and the percentage of the canopy cover was estimated for each plot with the help of these grid lines. Soil moisture and pH in each plot were recorded in the field with a calibrated pH and moisture reader electrode (Takemura Electric Soil Tester). The equipment gave the moisture reading on 0 to 8 scales, with the zero denoting the absence and 8 denoting the highest amount of soil moisture. We took pH and moisture reading from the four corners and center of each plot and then finally the averaged recorded values of soil pH and moisture for each plot. Furthermore, for estimating percentage of soil organic carbon, soil moisture, and soil pH in the laboratory, soil samples were collected from the four corners and one sample from the center (1-15 cm depth) of each plot. These subsamples were then mixed and meshed through a 2 mm sieve, and about 25 g were taken, air dried in shade and stored in airtight polythene bags for laboratory analysis. Such soil samples were collected from each plot during pre- as well as post-monsoon season and were analyzed at Central Department of Botany, Tribhuvan University, Kirtipur, Nepal. Percentage of soil organic carbon was estimated by Walkley and Black's rapid titration method as described in Black (1965). In the laboratory, pH from the saturated soil paste of each sample was recorded using a properly calibrated digital pH meter (pHep/ Hi 98107), following the method described in Gupta (2002). Percentage of soil moisture was estimated using the difference in the weight of the air-dried soil samples before and after storing these in the hot air woven at 160 ^{o}C for 24 hours. Finally, lab-recorded and field-recorded values of soil pH and soil moisture for each sampling season were averaged and regarded as final reading. The pattern of grazing in the study area was quite homogeneous in all kind of habitats. Therefore, we excluded grazing factor from our analyses.

Data analysis: We performed Detrended Correspondence Analysis (DCA) for the species datasets. The gradient length obtained from DCA was used to describe species heterogeneity, i.e., beta diversity (amount of compositional turnover in terms of Standard Deviation units) (Lepš & Šmilauer, 2003) along ecotone and also to determine the appropriate type of ordination analysis to be used to study species composition. As the gradient was very long (5.27 SD units), we assumed unimodal relationship between species and environmental variables and performed Canonical Correspondence Analysis (CCA) of species dataset. To elucidate the species-environment relationship, we used forward section method to select the significant environmental variables. The significance was tested by performing Monte Carlo permutation test. In the analysis, we removed species with less than three occurrences to reduce the negative influence of rare species on the results. All tests were carried out using Canoco 5.01 (ter Braak & Šmilauer, 2012).

To see if the species richness and environmental variables differed between vegetation type and depended on position along the transect, we used linear regression in S-Plus (S-PLUS, 2000). Duncan's post-hoc test comparison was performed to compare the categories and the figures were drawn using STATISTICA (StatSoft, 2004). We performed species response curve analysis for calculation of optima and tolerance values for each species. The width of the unimodal response curves (i.e. the tolerance) can be regarded as the realized niche (amplitude) of the species, and maximum value (optima) for the curve can be regarded as the indicator value of the species for that particular variable (ter Braak & Gremmen, 1987; Lepš & Šmilauer, 2003; Austin, 2005; Austin, 2007). We used generalized linear models (GLM) with logit link function, and fitted quadratic function of the predictors assuming binomial distribution of the errors by using logit link function. The species response curve test was also performed by using Canoco 5.01 (ter Braak & Šmilauer, 2012). We used only the species with more than eight occurrences for the response curve analysis.

Results

Species richness and compositional change: Altogether 66 herbaceous vascular plant species were recorded in the study site. Out of these, 59 species were identified to species level, six species to generic level, and two species only to family level. A total of 39 herb species were recorded in grassland, followed by 34 species in shrubland, 22 species in young forest, and 19 species in old forest in the study area (Table 1). The species richness significantly decreased from the grassland to the forests (Table 2, Fig. 2).

The DCA analysis showed a discrete distribution of herb species in different types of vegetation (viz. grassland-shrubland-young forest-old forest) along ecotone (Fig. 3). The CCA analysis indicated that the effect of habitat type was significant (p = 0.002, F-ratio = 15.43) and it explained 37.90% of the total variation of the dataset. The effect of habitat type on species composition was stronger than the effect of distance, where distance along the transect accounted only for 19.01% of total dataset (p = 0.002, F ratio = 18.30).

The species prevailing in grassland vegetation were e.g., *Erigeron multiradiatus*, *Gentiana argentea*, *Geranium procurrens and Kobresia* sp. Similarly *Artemisia gmelinii*, *Galium aparine*, and *Stellaria patens* mostly preferred shrubland. *Geranium donianum* was mainly found in young forest whereas *Cremanthodium hookeri*, *Galium paradoxum*, *Hemiphragma heterophyllum*, *Rubia wallichiana*, *Rubus nepalensis*, *Panax pseudo-ginseng* and *Thalictrum chelidonii* were found growing in old forest (Fig. 4). When choosing the most significant environmental variables after using habitat type as a covariate by forward selection method in CCA analysis, only canopy cover and organic carbon significantly influenced the distribution of herb species in the ecotone. Both the variables explained a total of 4.1% of the total variation of the dataset. The species preferring higher canopy cover were *Bistorta amplexicaulis*, *Halenia eilliptica*, *Persicaria polystachya*, *Thalictrum cultratum*, and *Viola biflora*; whereas *Anemone rivularis*, *Centella asiatica*, *Gentiana argentea*, *Kobresia* sp., *Trigonella emodi*, and *Gerbera nivea* preferred lower canopy cover. Similarly, *Rubia wallichiana*, *Smilax elegans*, *Smilacina purpurea* and *Taraxacum officinale* preferred organic carbon rich habitat; whereas *Campanula pallida*, *Dipsacus inermis*, *Euphrasia himalayica* and *Galium asperine* preferred lower organic carbon habitat (Fig. 5).

Environmental variables across vegetation types: Canopy cover, organic carbon, and soil moisture revealed significantly increasing trend whereas pH level decreased significantly from grassland to old forest (Fig. 6a-6d). Variations in the environmental factors were better reflected in the overall habitat conditions (= habitat types) than in the position along the transect (= spatial distance of plot along transect) in the univariate analyses (see Table 2). The different environmental variables were highly correlated ($R^2 > 0.84$ in all cases); organic carbon, soil moisture, and canopy were positively correlated whereas pH was negatively correlated with all the three environmental variables.

Species response to environmental variables: The environmental variables were highly correlated and forward selection of multivariate analysis showed significant effects only of canopy cover and soil organic carbon on composition of herb species in the ecotone. Therefore, we have shown the response analysis results for only these two environmental variables. When selecting the ten species with least tolerance value to each of the two environment variables, all the species except *Dipsacus inermis* and *Galium paradoxum*, responded significantly to both environmental variables. Altogether five least tolerance species were restricted to the old forest floor and three species each to the transition vegetation (shrubland and the young forest) and to the grassland (Appendix 1). Responses of *Anaphalis triplinervis*, *Anemone rivularis*, *Elsholtzia eriostachya*, *Geranium procurrens*, and *Lotus corniculatus* to both the environmental variables could not be estimated by the GLM procedure due to their variable responses (other than unimodal) to the environmental parameters. Response curve analysis of canopy cover showed the tolerance values from 1.14 (*Gentiana argentea*) to 13.13 (*Dipsacus inermis*). The species with least tolerance value in response analysis for canopy cover were *Dipsacus inermis*, *Gentiana argentea*, *Geranium donianum*, *Geranium* sp., *Kobresia* sp., *Potentilla griffithii*, *Rubus nepalensis*, *Rubia wallichiana*, *Thalictrum chelidonii*, and *Thalictrum cultratum* (Table 1, Fig. 7a). All the selected species showed a significant positive response to canopy cover ($p<0.001$ for each species). Out of the total species, *Gentiana argentea* and *Rubia wallichiana* respectively has the lowest (4.08) and the highest (75.71) optimum values for canopy cover (Table 1).

Response curve analysis of soil organic carbon showed the tolerance values from 0.17 (*Thalictrum cultratum*) to 0.50 (*Galium paradoxum*). Each of the species with localized distributions responds significantly to the soil organic carbon (each species with $p<0.001$). Among the potential organic carbon indicator species, *Potentilla griffithii* and *Galium paradoxum* have the lowest (3.09) and the highest (7.87) optimum values respectively. All the species other than the mentioned narrow-amplitude species are found to responding comparatively gradually to canopy cover and soil organic carbon. The species with the lowest tolerance values for organic carbon were *Galium paradoxum, Gentiana argentea, Geranium donianum, Geranium* sp., *Kobresia* sp., *Potentilla griffithii*, *Rubus nepalensis, Rubia wallichiana, Thalictrum chelidonii* and *Thalictrum cultratum* (Table 1, Fig. 7b).

Table 1. Herbaceous species recorded from different vegetation types along ecotone and the tolerance values of the frequent species (species having more than eight occurrences) for different environmental parameters.

S.N.	Full name of species	Abbreviation	Vegetation type	Optima values		Tolerance values	
				Canopy	OC	Canopy	OC
1.	*Allium wallichii* Kunth	All.wal	Sh, Yf	NA	NA	NA	NA
2.	*Anaphalis triplinervis* (Sims) C. B. Clarke	Ana.tri	Gr, Sh, Of	NA	NA	NA	NA
3.	*Androsace strigillosa* Franch.	Andro.st	Sh	NA	NA	NA	NA
4.	*Anemone rivularis* Buch.-Ham. ex DC.	Ane.riv	Gr, Sh, Yf, Of	NA	NA	NA	NA
5.	*Arisaema propinquum* Schott	Aris.pr	Of	NA	NA	NA	NA
6.	*Artemisia gmelinii* Weber ex Stechm.	Art.gm	Gr, Sh, Yf	9.25	3.81	25.81	1.34
7.	*Astilbe rivularis* Buch.-Ham ex D. Don	Ast.riv	Yf	NA	NA	NA	NA
8.	*Bistorta amplexicaulis* (D. Don) Greene	Bist.am	Gr, Sh	NA	NA	NA	NA
9.	*Calamogrostis* sp.	Calamo	Gr	NA	NA	NA	NA
10.	*Campanula pallida* Wall.	Camp.pal	Gr	NA	NA	NA	NA
11.	*Centella asiatica* (L.) Urb.	Cent.as	Gr, Sh	NA	NA	NA	NA
12.	*Chenopodium foliosum* (Moench) Asch.	Chen.fo	Gr	NA	NA	NA	NA
13.	*Cirsium falconeri* (Hook.f.) Petr.	Cir.fal	Yf	NA	NA	NA	NA
14.	*Corydalis* sp.	Corydal	Yf	NA	NA	NA	NA
15.	*Cremanthodium hookeri* C. B. Clarke	Crem.ho	Of	106.20	7.98	17.99	0.61
16.	*Cynodon dactylon* (L.) Pers.	Cyn.dac	Gr, Sh	-27.01	3.43	24.70	0.55
17.	* *Dipsacus inermis* (Wall)	Dips.in	Gr, Sh, Yf	37.11	5.07	13.13	0.60
18.	*Elsholtzia eriostachya* (Benth.) Benth.	Els.eri	Gr, Sh	NA	NA	NA	NA
19.	*Erigeron multiradiatus* (Lindl. ex DC) C. B. Clarke	Eri.mul	Gr, Sh	-721.46	1.07	96.09	1.20
20.	*Euphrasia himalayica* Wettst.	Euph.him	Gr, Sh, Yf	17.22	3.37	21.99	1.61
21.	*Fagopyrum tataricum* (L.) Gaertn.	Fago.ta	Yf	NA	NA	NA	NA
22.	*Fragaria nubicola* Lindl. ex Lacaita	Frag.nub	Gr, Sh, Yf, Of	38.87	5.51	14.01	0.80
23.	*Galium aparine* L.	Gal.asp	Gr, Sh, Yf	27.30	4.62	13.91	0.88
24.	* *Galium paradoxum* Maxim.	Gal.par	Of	99.29	7.87	14.85	0.50
25.	** *Gentiana argentea* (D. Don) C. B. Clarke	Gen.arg	Gr	4.08	3.15	1.14	0.19
26.	** *Geranium donianum* Sweet	Ger.don	Yf	41.44	5.57	11.40	0.30
27.	*Geranium procurrens* Yeo	Ger.pro	Gr, Sh	NA	NA	NA	NA
28.	** *Geranium* sp.	Gerani	Of	74.2	7.51	10.66	0.31
29.	*Gerbera nivea* (DC.) Sch. Bip.	Gerb.niv	Gr, Sh	NA	NA	NA	NA
30.	Gramineae sp	Gramine	Sh, Yf	43.6	5.47	10.72	0.55
31.	*Gypsophila cerastioides* D. Don	Gyp.cer	Gr, Sh	-72.94	3.31	49.79	0.82
32.	*Halenia elliptica* D. Don	Hal.ell	Gr, Sh	NA	NA	NA	NA
33.	*Hemiphragma heterophyllum* Wall.	Hemi.het	Yf, Of	91.43	15.11	13.29	1.96
34.	*Heracleum candicans* Wall. ex DC.	Hera.can	Gr, Sh	18.92	3.89	16.37	0.59
35.	*Impatiens radiata* Hook. f.	Impa.ret	Yf, Of	65.19	6.65	16.08	0.57
36.	*Iris kemaonensis* D. Don ex Royle	Iris.ke	Sh	NC	NC	NC	NC
37.	** *Kobresia* sp.	Kobres	Gr	4.80	3.20	1.53	0.27
38.	*Lindelofia anchusoides* (Lindley) Lehm.	Lin.anc	Yf	NA	NA	NA	NA
39.	*Lotus corniculatus* L.	Lot.cor	Gr, Sh	NA	NA	NA	NA
40.	*Oenothera* sp.	Oenothe	Of	100.53	7.84	16.99	0.58
41.	*Oxytropis microphylla* (Pall.) DC.	Oxyt.mic	Gr	NA	NA	NA	NA
42.	*Panax pseudo-ginseng* Wall.	Panax.ps	Of	NA	NA	NA	NA
43.	*Parochetus communis* Buch.-Ham. ex D. Don	Paro.com	Gr	NA	NA	NA	NA
44.	*Pedicularis gracilis* Wall. ex Benth.	Pedi.gr	Gr, Sh	NA	NA	NA	NA
45.	*Peristylus fallax* Lindl.	Peri.sup	Gr	NA	NA	NA	NA
46.	*Persicaria polystachya* (Wall. ex Meisn.) H. Gross	Perscari	Gr, Sh, Yf, Of	33.93	4.93	20.36	1.29
47.	*Plantago erosa* Wall.	Plan.er	Gr	NA	NA	NA	NA
48.	*Polygonatum cirrhifolium* (Wall.) Royle	Poly.cir	Gr, Sh	12.68	3.67	16.02	0.57
49.	*Potentilla atrosanguinea* (Lodd.) Hook.f.	Pote.at	Sh	NA	NA	NA	NA
50.	** *Potentilla griffithii* Hook.f.	Pot.gri	Gr	4.80	3.09	1.53	0.31
51.	*Roscoea alpina* Royle	Rosc.al	Gr, Sh	-30.99	3.30	26.85	0.60
52.	** *Rubia wallichiana* Decne	Rubi.wal	Of	75.71	7.59	9.48	0.45
53.	** *Rubus nepalensis* (Hook.f.) Kuntze	Rub.nep	Of	73.37	7.45	8.17	0.37
54.	*Rumex nepalensis* Spreng.	Rum.nep	Gr, Sh, Yf	22.91	4.53	13.91	1.26
55.	*Saussurea fastuosa* (Decne.) Sch. Bip.	Sau.fas	Gr	NA	NA	NA	NA
56.	*Selinum wallichianum* (DC.) Raizada & Saxena	Seli.wal	Gr, Sh, Yf	14.90	4.09	21.54	0.80
57.	*Smilacina purpurea*Wall.	Smi.pur	Of	NA	NA	NA	Na
58.	*Smilax elegans* Wall. ex Kunth	Smil.el	Of	NA	NA	NA	NA
59.	*Stellaria patens* D. Don	Ste.pat	Gr, Sh, Yf	22.92	4.21	15.48	1.02
60.	*Taraxacum officinale* F. H. Wigg.	Tara.of	Gr, Sh	NA	NA	NA	NA
61.	** *Thalictrum chelidonii* DC.	Thal.ch	Yf, Of	74.97	7.16	12.25	0.42
62.	** *Thalictrum cultratum* Wall.	Thal.cul	Sh	35.46	4.65	11.95	0.17
63.	*Trigonella emodi* Benth.	Tri.emo	Gr, Sh	NA	NA	NA	NA
64.	*Typhonium diversifolium* Wall. ex Shott	Typ.div	Gr	NA	NA	NA	NA
65.	*Urtica* sp.	Urtica	Of	NA	NA	NA	NA
66.	*Viola biflora* L.	Viol.bi	Sh, Yf, Of	63.44	7.28	22.02	1.58

Ten lowest tolerance values are in bold letters; Gr = grassland, Sh = shrubland, Yf = young forest, Of = Old forest; OC = soil organic carbon. *Species having significant response to the environmental factors (each of the selected species having $p<0.001$ for each of the environmental factor) and covering less than one fourth of the whole gradient length; **species having significant response and narrow niche to both the environmental variables. NA = not calculated due to being rare (< 8 occurrences) or could not be estimated by analysis due to having other responses than the unimodal.

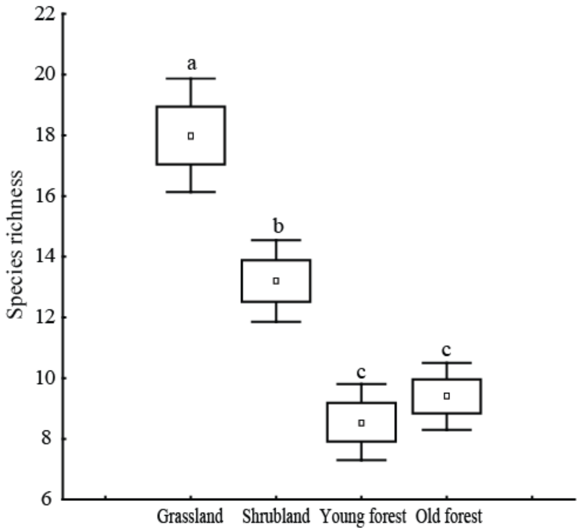


Fig. 2. Species richness in different types of vegetation. The graph shows mean, SE and 1.96 SE. N = 80 in each category.

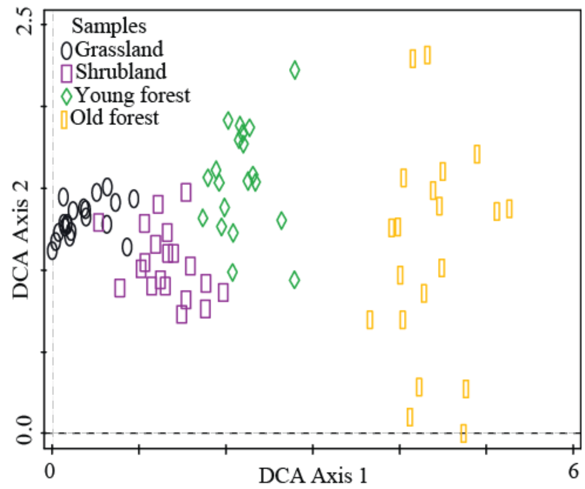


Fig. 3. DCA biplot showing distribution of herb species in grassland-shrubland-young forest-old forest in Langtang valley, central Nepal. The 1st canonical axis explained 22.99% and the 2nd explained 3.7% of the total variation of the data set.

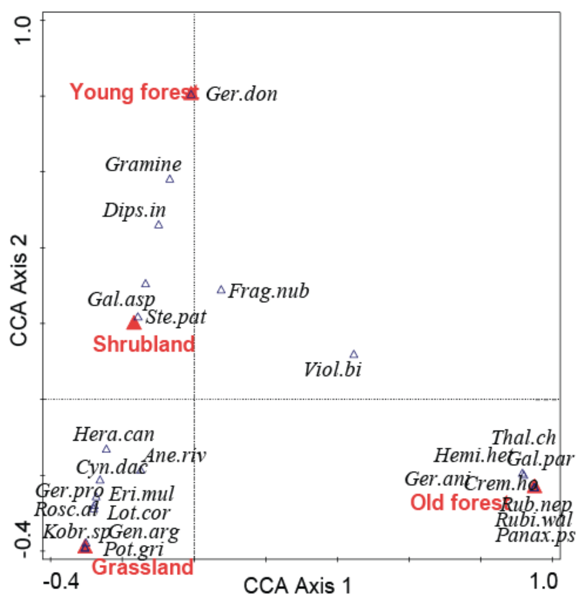


Fig. 4. CCA biplot showing effect of locality in distribution of herb species along the ecotone in Langtang valley, central Nepal. First axis explains 21.29% and the second axis explains 12.38% of the total variation in the dataset.

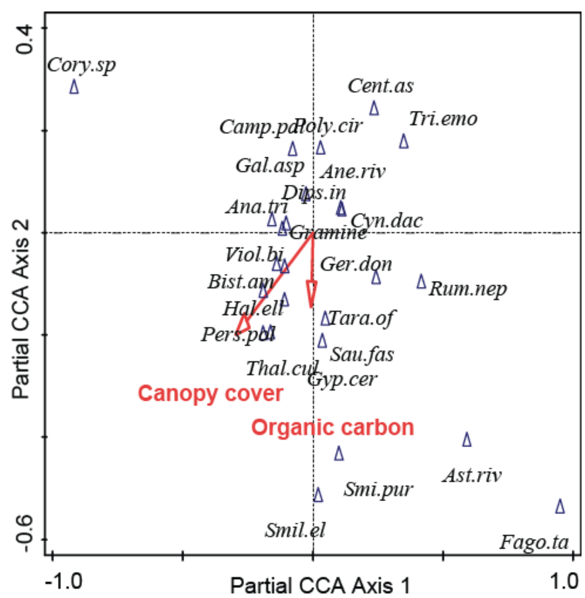
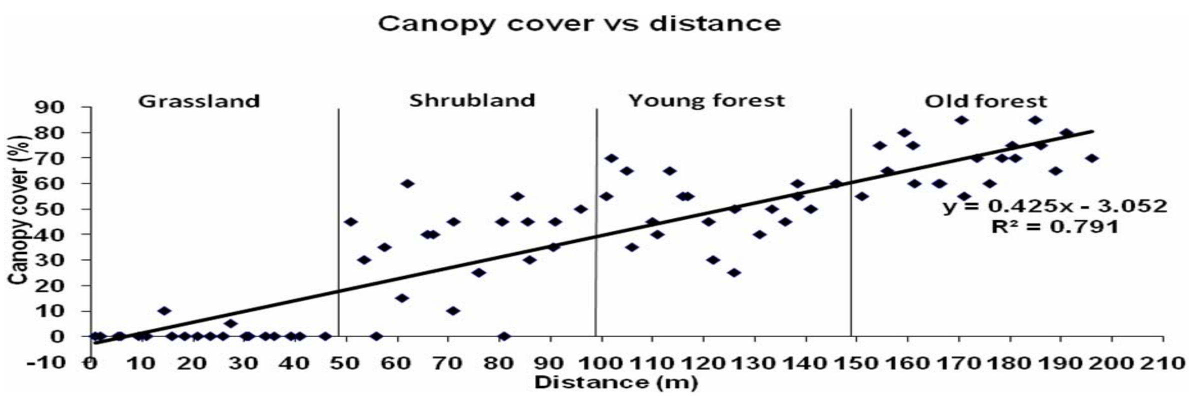


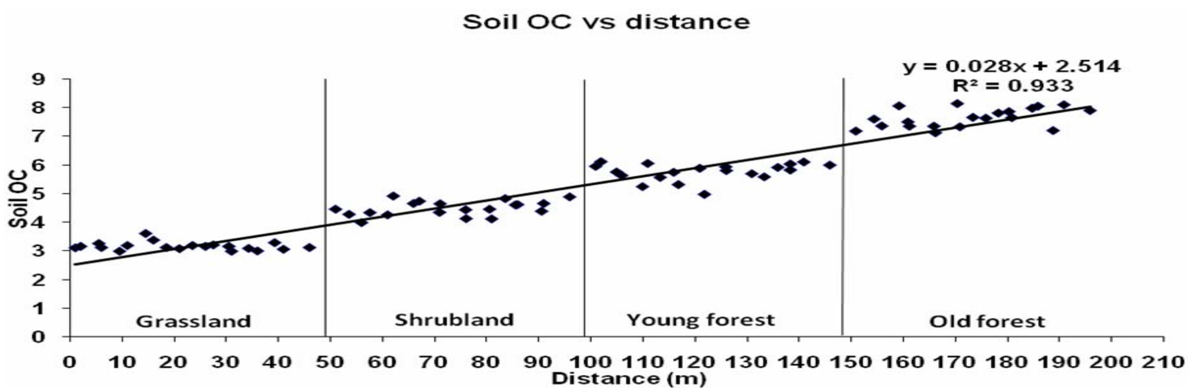
Fig. 5. CCA biplot showing the relationship between herb species and canopy cover of the plant species in grassland-shrubland-young forest-old forest in Langtang valley, central Nepal. Only the first axis explains 2.23% and the second axis explains 1.49% of the total variation in the dataset.

Table 2. Effect of vegetation types (df = 3) and distance of along sampled transects (df = 1) in ecotone vegetation on species richness, canopy cover and soil characteristics.

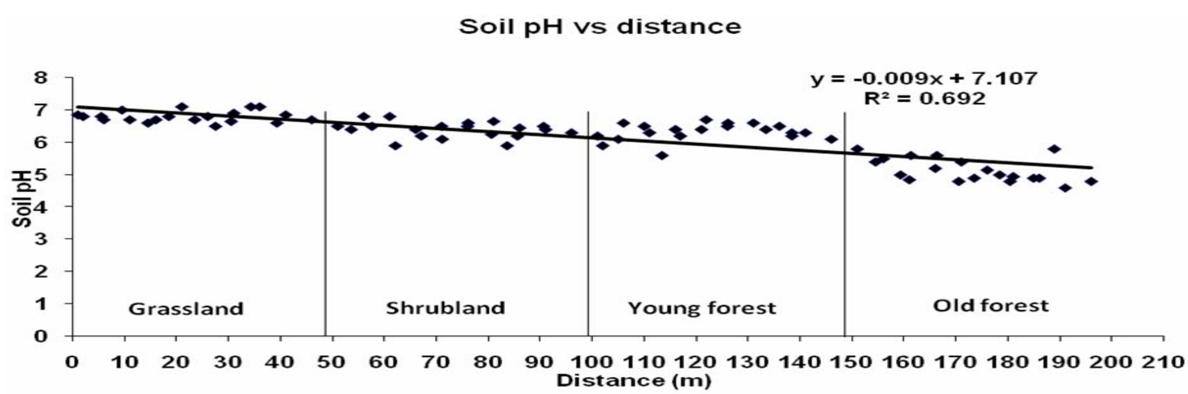
Dependent variables	F value	Pr(F)	R sq	F value	Pr(F)	R sq
Species richness	35.28	<0.001	0.58	61.96	<0.001	0.44
Canopy cover	129.4	<0.001	0.83	296.28	<0.001	0.79
Organic carbon	999.48	<0.001	0.97	1089.83	<0.001	0.93
Soil moisture	175.88	<0.001	0.87	364.03	<0.001	0.82
Soil pH	136.52	<0.001	0.84	175.84	<0.001	0.69



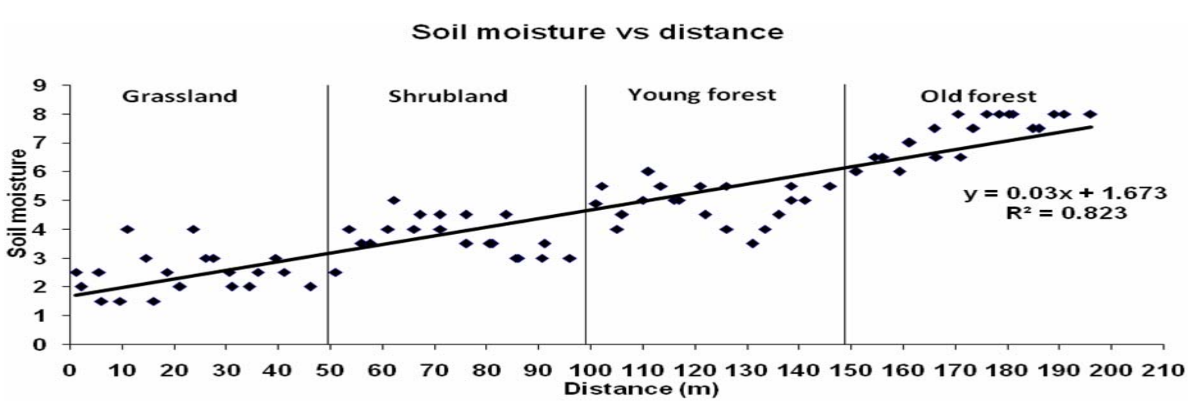
(a) Variation of canopy cover along the transect



(b) Variation of soil organic carbon along the transect



(c) Variation of soil pH along the transect



(d) Variation of soil moisture along the transect

Fig. 6a-6d. Variation pattern of the environmental parameters in grassland-shrubland-young forest-old forest ecotone vegetation along transect (m = meters).

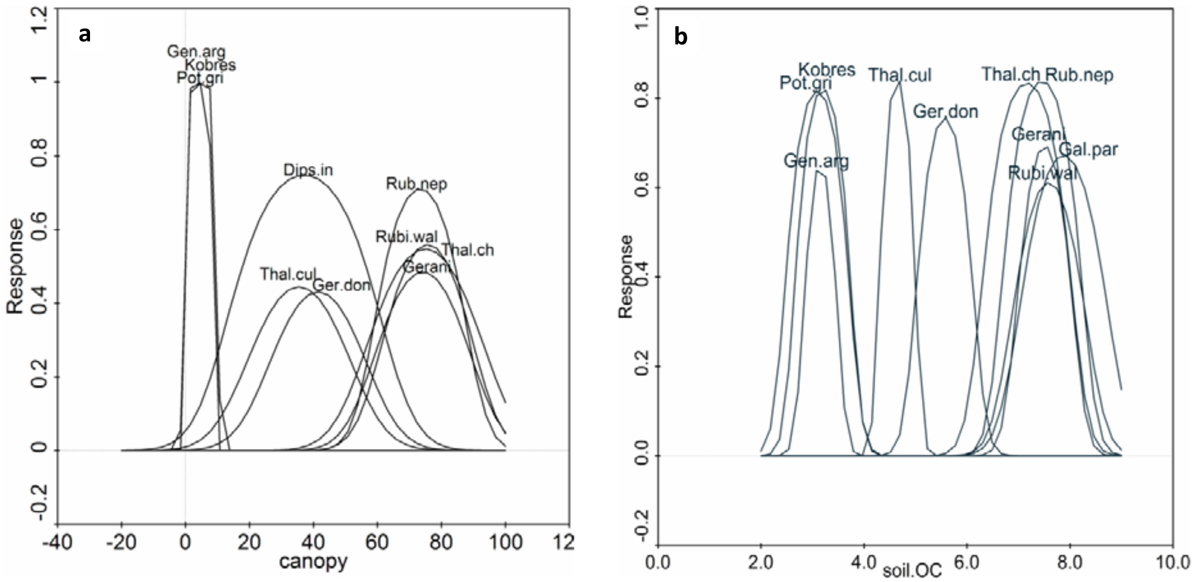


Fig. 7a-7b. Response curves of the narrow-amplitude species to (a) canopy cover, (b) soil organic carbon (soil.OC); curves fitted in CanoDraw by a second-order polynomial predictor (GLM procedure; $p<0.00001$ for each species). Dips.in = *Dipsacus inermis*, Gal.par = *Galium paradoxum*, Gen.arg = *Gentiana argentea*, Gerani = *Geranium* sp., Ger.don = *Geranium donianum*, Gramine = Graminoid, Kobres = *Kobresia* sp., Pot.gri = *Potentilla griffithii*, Rubi.wal = *Rubia wallichiana*, Rub.nep = *Rubus nepalensis*, Thal.ch = *Thalictrum*

Discussion

Species richness and compositional change: The changing pattern in richness and composition of herbaceous species from the open grassland to an old forest in general is similar to the findings of Shrestha and Vetaas (2009) from an alpine forest ecotone of Manang district, Nepal. The gradient length in our study was higher (5.27 SD units) than Shrestha & Vetaas (2009) (vertical transects = 2.6 SD and horizontal transects =1.8 SD) meaning that species turnover (beta diversity) of the herbaceous species was significantly higher in our study. However, there was low species richness in the young forest compared to other habitats. The young forest is newly grown on the glacial debris and such debris is unfavourable for colonization of herbaceous species. The steep gradient length in DCA of our dataset is due to the profound floristic dissimilarity among the sample plots of ecotone as mentioned by Lepš & Šmilauer (2003). However, from grassland to the young forest, variation in the environmental factors is gradual in comparison to that in the old forest. Therefore, the plots from the three vegetation types are clumped closer. There is a considerable difference in the soil factors as well as canopy cover between the old forest and the rest of the vegetation types. This might have restricted most of the forest herbs only to the old forest floor. Such a high floristic dissimilarity isolated the old forest plots distinctly from the rest of the vegetation types in the ordination space. The DCA biplot showed that most of the variance is explained by the first DCA axis and the succeeding axes explain negligible amount of the variation, indicating redundant secondary gradient.

The change pattern of herbaceous species between habitat types can partly be attributed to the changes in soil organic carbon and canopy cover. Soil organic carbon is regulated by litter decomposition and metabolism and strongly affected by the presence and type of tree layer (Amatangelo *et al.*, 2008). This variation of litter leads to differentiation of soil properties thus leading to distinct composition pattern in various habitat types in ecotone. Litter accumulation in the forest floor and gaps in the canopy caused by falling of single tree, or even large tree limbs may also create diversity in micro-environmental conditions just a few centimeters or meters in distance (Lomolino *et al.*, 2006; Stinchcombe & Schmitt, 2006). Canopy cover maintains soil microclimate by regulating the quantity and quality of light penetration to the forest floor, increasing soil moisture, and reducing temperatures (Armand, 1992; Jensen & Gutekunst, 2003; Amatangelo *et al.*, 2008). This affects the growth and survival of understory herb plant species. On the other hand, variation of diurnal temperature occurs more in the open grasslands than that in the dense canopy forests. It may favor the growth of higher number of herb species towards grassland than in the forest (Grytnes & Birks, 2003). In addition to this, disturbance caused by moderate grazing frequencies/ intensities of cattle in the grassland and shrubland possibly play a vital role in maintaining higher species richness in the open land by maintaining habitat heterogeneity (Vandvik *et al.*, 2005; Alados *et al.*, 2007), despite its negative consequences towards soil organic carbon and nitrogen concentrations (Feng *et al.*, 2015).

Environmental variables across vegetation types: In our study, variation of each of the environmental variables along the transect is found to be better explained by the habitat types than by the position of the plots along the transect. This suggests that overall variations in the environmental conditions across ecotone are at least partly induced by the conditions of individual habitat type along ecotone. Since we have four distinct (physiognomically differentiated) habitat types in the ecotone, having their own specific biotic and abiotic components and various interactions among those components, abrupt change in the overall environmental conditions along the transect was observed.

In the old forest, more litter and thus humus accumulation, in comparison to other adjacent habitat types, is expected leading to acidification and increased moisture availability. Such effect has been demonstrated in forests dominated by gymnosperms (Sharma, 2003). In addition, the moisture availability is also clearly linked to canopy cover, which itself is a direct reflection of vegetation type (Klanderud, 2008).

Species responses to the environmental parameters: Many studies have demonstrated that the species distribution is determined mainly by environmental variables such as soil nutrients, and climatic factors (Austin, 2007). Therefore, we took an account of three edaphic factors (soil pH, soil moisture, and soil organic carbon), and canopy cover (surrogate of sunlight). All these factors contribute in creating microenvironment of the plant species that influence their survival, growth, and local dispersal (Körner, 1999; Naqinezhad *et al.*, 2013).

Response curve analysis revealed the individualistic response of species to each of the selected environmental factor. Despite the differences in shapes of the response curves of species in different analyses, curves for the species with narrow niche are mostly the unimodal in shape in our study because these species are frequent but cover only a small fraction of the long gradient (Rydgren *et al.*, 2003; Peppler-Lisbach, 2008). The species response curve along an environmental gradient in the presence of other species is equivalent to the realized utilization function for habitat or environmental niche (Whittaker *et al.*, 1973; Austin, 1985; Austin, 2005). The width of the unimodal response curves (i.e. the tolerance) are also regarded as the realized niche (amplitude) of the species, and maximum value (peak) for the curve can be regarded as the indicator value of the species for that particular variable (ter Braak & Gremmen, 1987; Lepš & Šmilauer, 2003).

Findings from ordination and regression analyses revealed that species those are less dominant and are with a narrow range are distributed abruptly along the environmental gradient as suggested by Gauch & Whittaker (1972). Species confined to a particular habitat vary abruptly with changes in habitat type and such species also have a very narrow niche (Fig. 7). We have selected the ten species with the least tolerance values, each of those occupying less than one fourth of the whole gradient, as 'narrow-amplitude species'. Based upon the criteria, almost the same species (in type and number) were found to be sensitive to canopy cover and soil OC. This indicates both soil OC and sunlight as the governing factors of the herbaceous species composition as suggested by many studies (Foumier & Planchon, 1998; Jensen & Gutekunst, 2003; Eckstein & Donath, 2005; Kostel-Hughes *et al.*, 2005; Amatangelo *et al.*, 2008; Klanderud, 2008).

Conclusion

Herbaceous species of the subalpine ecotone are mostly influenced by canopy cover and soil organic carbon. Species such as *Gentiana argentea, Geranium donianum, Kobresia* sp., *Potentilla griffithii, Rubia wallichiana, Rubus nepalensis, Thalictrum chelidonii* and *Thalictrum cultratum* are supposed to be critically sensitive to local environmental conditions due to having narrow amplitude (the least tolerance) to both the analyzed environmental variables. Although we have single-time data set from the sole existing horizontal ecotone in the National Park, these indicator species could be vital for long-term assessment of the critical ecotone vegetation, especially in the present context of climate and land use- changes. Broader-scale replicated studies regarding these species would have a greater prospect in understanding climate- and land use- change mediated community dynamics of all the subalpine ecotones.

Acknowledgements

This research was conducted as part of the master degree in Biodiversity and Environmental Management, supported by NOMA – Norad's Programme for Master Studies. K.P. Bhatta is thankful to Prof. Ole Reidar Vetaas and Prof. Ram Prasad Chaudhary for their supervision of the Master's research. Thanks to Mr. Madan K. Suwal for the preparation study area map.

References

Alados, C.L., A.E. Aich, B. Komac, Y. Pueyo and R. García-Gonzalez. 2007. Self organized spatial patterns of vegetation in alpine grasslands. *Ecol. Model.*, 201: 233-242.

Amatangelo, K.L., J.S. Dukes and C.B. Field. 2008. Responses of a california annual grassland to litter manipulation. *J. Veg. Sci.*, 19(5): 605-612.

Anonymous. 2013. IPCC. Climate change: The physical science basis. Contribution of working group i to the fifth assessment report of the intergovernmental panel on climate change. Stocker, T.F.D. QinG.-K. Plattner*et al.* (Eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Armand, A. 1992. Sharp and gradual mountain timberlines as a result of species interaction. *In*: Hansen, A. and F. Castri (Eds.), *Landscape boundaries*. Springer New York, pp. 360-378.

Austin, M.P. 1985. Continuum concept, ordination methods, and niche theory. *Annu. Rev. Ecol. Syst.*, 16: 39-61.

Austin, M.P. 2005. Vegetation and environment: Discontinuities and continuities. *In*: van der Maarel, E. (Ed.), *Vegetation ecology*. Blackwell Publishing, Oxford, pp. 52-84.

Austin, M.P. 2007. Species distribution models and ecological theory: A critical assessment and some possible new approaches. *Ecol. Model.*, 200: 1–19.

Black, C.A. (Ed.) 1965. *Methods of soil analyses part 1 and 2*. American Society of Agronomy. Maidson, USA.

D'Odorico, P., Y. He, S. Collins, S.F.J. De Wekker, V. Engel*, et al.* 2013. Vegetation–microclimate feedbacks in woodland–grassland ecotones. *Global Ecol. Biogeogr.*, 22(4): 364-379.

Diekmann, M. 2003. Species indicator values as an important tool in applied plant ecology – a review. *Basic Applied Ecology*, 4: 493-506.

Dunnett, N.P. and J.P. Grime. 1999. Competition as an amplifier of short-term vegetation responses to climate: An experimental test. *Funct. Ecol.*, 13: 388-395.

Eckstein, R.L. and T.W. Donath. 2005. Interactions between litter and water availability affect seedling emergence in four familial pairs of floodplain species. *J. Ecol.*, 93: 807-816.

Ellenberg, H., H.E. Weber, R. Düll, V. Wirth, W. Werner*, et al.* 1991. Zeigerwerte von pflanzen in mitteleuropa. *Scripta Geobotanica*, 18: 1-248.

Eppinga, M.B., C.A. Pucko, M. Baudena, B. Beckage and J. Molofsky. 2013. A new method to infer vegetation boundary movement from 'snapshot' data. *Ecography*, 36: 622-635.

Feng, J., X. Han, N. He, Y. Zhang, L. Zhou*, et al.* 2015. Sheep grazing stimulated plant available soil nitrate accumulation in a temperate grassland. *Pak. J. Bot.*, 47(5): 1865-1874.

Foumier, A. and O. Planchon. 1998. Link of vegetation with soil at a few metre-scale: Herbaceous floristic composition and infiltrability in a sudanian fallow-land. *Acta Oecol.*, 19(3): 215-226.

Gauch, H.G. and R.H. Whittaker. 1972. Coenocline simulation. *Ecology*, 53: 446-451.

Gégout, J.-C., J.-C. Hervé, F. Houllier and J.-C. Pierrat. 2003. Prediction of forest soil nutrient status using vegetation. *J. Veg. Sci.*, 14: 55-62.

Gigauri, K., M. Akhalkatsi, G. Nakhutsrishvili and O. Abdaladze. 2013. Monitoring of vascular plant diversity in a changing climate in the alpine zone of the central greater caucasus. *Turk. J. Bot.*, 37: 1104-1114.

Gottfried, M., H. Pauli and G. Grabherr. 1998. Prediction of vegetation patterns at the limits of plant life: A new view of the alpine-nival ecotone. *Arct. Alp. Res.*, 30(3): 207-221.

Grytnes, J.A. and H.J.B. Birks. 2003. The influence of scale and species pool on the relationship between vascular plant species richness and cover in an alpine area in norway. *Plant Ecol.*, 169: 273-284.

Gupta, P.K. 2002. *Methods in environmental analysis: Water, soil and air*. Agrobios.

Hartley, I.P., M.H. Garnett, M. Sommerkorn, D.W. Hopkins, B.J. Fletcher*, et al.* 2012. A potential loss of carbon associated with greater plant growth in the european arctic. *Nature Clim. Change*, 2(12): 875-879.

Hill, M.O., J.O. Mountford, D.B. Roy and R.G.H. Bunce. 1999. *Ellenberg's indicator values for british plants. Ecofact volume 2 technical annex*. Institute of Terrestrial Ecology, Huntingdon.

Jensen, K. and K. Gutekunst. 2003. Effects of litter on establishment of grassland plant species: The role of seed size and successional status. *Basic Appl. Ecol.*, 4(6): 579-587.

Khan, S.M., S. Page, H. Ahmad, Zahidullah and H. Shaheen. 2013. Phyto-climatic gradient of vegetation and habitat specificity in the high elevation western himalayas. *Pak. J. Bot.*, 45(SI): 223-230.

Klanderud, K. 2008. Species-specific responses of an alpine plant community under simulated environmental change. *J. Veg. Sci.*, 19(3): 363-372.

Körner, C. 1999. *Alpine plant life: Functional plant ecology of high mountain ecosystems*. Spring Verlag, Berlin.

Kostel-Hughes, F., T.P. Young and J.D. Wehr. 2005. Effects of leaf litter depth on the emergence and seedling growth of deciduous forest tree species in relation to seed size. *The Journal of the Torrey Botanical Society*, 132: 50-61.

Lepš, J. and P. Šmilauer. 2003. *Multivariate analysis of ecological data using canoco*. Cambridge University Press, UK.

Lindborg, R., S.A.O. Cousins and O. Eriksson. 2005. Plant species response to land use change - *campanula rotundifolia*, *primula veris* and *rhinanthus minor*. *Ecography*, 28: 29-36.

Lomolino, M.V., B.R. Riddle and J.H. Brown. 2006. *Biogeography*. (3rd Ed) Sinauer Associates, Inc, Sunderland, Massachusetts.

Lutz, D.A., R.L. Powell and M.R. Silman. 2013. Four decades of andean timberline migration and implications for biodiversity loss with climate change. *PloS one*, 8(9): e74496.

Naqinezhad, A., H. Zare-Maivan, H. Gholizadeh and J.G. Hodgson. 2013. Understory vegetation as an indicator of soil characteristics in the hyrcanian area, n. Iran. *Flora*, 208: 3-12.

Pausas, J.G. and M.P. Austin. 2001. Patterns of plant species richness in relation to different environments: An appraisal. *J. Veg. Sci.*, 12: 153-166.

Peppler-Lisbach, C. 2008. Using species-environmental amplitudes to predict ph values from vegetation. *J. Veg. Sci.*, 19(4): 437-444.

Persson, S. 1981. Ecological indicator values as an aid in the interpretation of ordination diagrams. *J. Ecol.*, 69: 71-84.

Press, J.R., K.K. Shrestha and D.A. Sutton. 2000. *Annotated checklist of the flowering plants of nepal*. The Natural History Museum, London.

Richardson, D.M., R.M. Cowling, B.B. Lamont and H.J.v. Hensbergen. 1995. Coexistence of banksia species in southwestern australia: The role of regional and local processes. *J. Veg. Sci.*, 6: 329-342.

Rydgren, K., R.H. Økland and T. Økland. 2003. Species response curves along environmental gradients. A case study from se norwegian swamp forests. *J. Veg. Sci.*, 14: 869-880.

S-PLUS. 2000. Professional edition for windows, release 2. Mathsoft, inc.

Sharma, P.D. 2003. *Ecology and environment*. Rastogi Publications, India.

Shrestha, K.B. and O.R. Vetaas. 2009. The forest ecotone effect on species richness in an arid trans-himalayan landscape of nepal. *Folia Geobot.*, 44: 247-262.

Smart, S.M. 2000. Ecological assessment of vegetation from a nature reserve using regional reference data and indicator scores. *Biodivers. Conserv.*, 9: 811-832.

StatSoft, I. 2004. Statistica data analysis software system. Version 7. Statsoft, inc.

Stinchcombe, J.R. and J. Schmitt. 2006. Ecosystem engineers as selective agents: The effects of leaf litter on emergence time and early growth in impatiens capensis. *Ecol. Lett.*, 9: 255-267.

ter Braak, C.J.F. and N.J.M. Gremmen. 1987. Ecological amplitudes of plant species and the internal consistency of ellenberg's indicator values for moisture. *Vegetatio*, 69: 79-87.

ter Braak, C.J.F. and P. Šmilauer. 2012. *Canoco reference manual and user's guide: Software for ordination, canoco 5*. Biometris, Plant Research International, The Netherlands and Czech Republic.

Vandvik, V., E. Heegaard, I.E. Måren and P.A. Aarrestad. 2005. Managing heterogeneity: The importance of grazing and environmental variation on post-fire succession in heathlands. *J. Appl. Ecol.*, 42: 139-149.

Vetaas, O.R. and J.A. Grytnes. 2002. Distribution of vascular plant species richness and endemic richness along the himalayan elevation gradient in nepal. *Glob. Ecol. Biogeogr.*, 11: 291-301.

Wamelink, G.W.W., P.W. Goedhart, J.Y. Frissel, R.M.A. Wegman, P.A. Slim*, et al.* 2007. Response curves for plant species and vegetaion types. Alterra-rapport 1489/ juni / 2007; www.Alterra.Wur.Nl

Whittaker, R.H., S.A. Levin and R.B. Root. 1973. Niche, habitat and ecotope. *Am. Nat.*, 107: 321-338.

(Received for publication 25 November 2014)