BJNRD

BJNRD (2018), 4(2): 10-28
Bhutan Journal of Natural Resources & Development

Article

www.bjnrd.org

Open Access

ISSN 2409–2797 (Print)
ISSN 2409–5273 (Online)

https://doi.org/10.17102/cnr.2017.06

Grassland Communities, Graminoid Composition, and their Diversity Pattern on the Eastern Mountain Slope of Dochula, Bhutan

Tshering Dorji[1] and Dhan Bdr. Gurung[2]

Abstract

The composition of grassland communities in Bhutan is poorly understood. This study was conducted to determine the grassland communities and graminoid diversity pattern among different vegetation types on the east slope of Dochula. We laid twenty-four Modified Whittaker (MWP) nested plots in six different vegetation types through stratified random sampling. A total of 268 plant species were recorded, of which 110 were graminoids. Mean alpha richness ranged from 16–38 species between MWPs. Species richness and Shannon index were the highest in agriculture land, while meadow had the lowest. One-way ANOVA showed significantly higher species diversity and richness in agriculture land. Three grassland communities were identified through cluster analysis: Dry Chirpine grassland, Agriculture meadows, and Cool temperate grassland. This study explored the possibility to classify grassland communities in Bhutan and suggests that the current method can be upscaled to classify grassland communities at a national level. However, additional environmental factors such as soil moisture, soil temperature, and nutrient content are required to better explain species distribution and their interactions.

Keywords: Bhutan, graminoids, grassland, Modified-Whittaker Plot

Introduction

Graminoids encompass about 20,000 species and dominate major vegetation types in the world (Rawat, 1998). The Himalaya constitutes one of the richest and most unusual ecosystems on Earth (Salick *et al*., 2009), attributed mainly to the different forest types influenced by varying altitude, topographic, and climatic conditions (Mani, 1978). Bhutan, straddled between the Indomalayan and the Palearctic zones, has a rich biodiversity, with 5,603 recorded vascular plants (National Biodiversity Centre, 2014). Currently, 484 species of graminoids are recorded from Bhutan (Noltie, 2000).

Four of the five major grassland types of the Himalayas identified by Rawat (1998) are known to occur in the country. Further, Tshuchida (1987) identified four grassland zones as follows: Zone A (150–2500 m), Zone B (2500–3500 m); Zone C (3500–4000 m), and Zone D (4000–5000 m). The grass communities in eastern Bhutan according to Miller (1987) are *Cymbopogon* grassland; *Schyzachryium* grassland; *Danthonia* grassland, and *Kobresia/Carex* alpine meadow. According to Noltie (2000), some of the vegetation types rich in grasses include Terai, low-altitude river banks or flats

[1]National Biodiversity Centre, Serbithang, Ministry of Agriculture and Forests
[2]College of Natural Resources, Royal University of Bhutan, Lobesa
Corresponding author: tdorji1@moaf.gov.bt
Received Jul., 2017; Accepted Dec., 2017

(150–700 m), Chirpine forest (900–1800 m), cool temperate grasslands (2300–3000 m), sub-alpine grassland (3600–3000 m), and alpine pasture (over 4000 m).

Grasslands continue to be among the most threatened ecosystems in the world due to overgrazing, exotic species invasions, and woody encroachment (Gibson, 2009; Samson and Knopf, 1994). Despite this, there has been little impetus for the study of grassland communities in Bhutan. Much of the works in Bhutanese grasslands have been devoted to rangeland resources and management, particularly in the alpine region (Gyamtsho, 1996; Moktan *et al.,* 2008; Wangchuk *et al.*, 2013). Hence, our understanding of grassland communities in temperate Bhutan is limited (Roder *et al.,* 1998 as cited in Noltie, 2000).

This study was conducted to compare species richness and abundance of graminoids in different habitat types on the east-facing mountain slope of Dochula, and to classify grassland communities in the study area. Rawat (1998) recognized the need for studies in temperate grasslands, which harbor a wide range of flora and fauna. Also, works on the ecology and distribution of grass species remain a priority (Noltie, 2000). Forest grasslands support a wide range of services in Bhutan, such as food, pasture, raw materials, and religious purposes (Miller, 1987; Noltie, 2000). Therefore, a timely study on grassland communities is important for conservation and sustainable utilization, mainly to assist planners in preparing appropriate management plans.

Materials and Methods

Study area

The study was conducted on the east mountain slope of Dochula. The study area is located between 27° 29’ 24” N to 27° 28’ 43.69 N” and 089° 45’ 01” E to 089° 53’ 45.3” E. The area is a good example of a temperate forest with bottom dry valleys to top humid mountains encompassing altitude between 1250–3100 masl (Wangda and Ohsawa, 2006). Due to the altitudinal difference of almost 2000 m, climatic condition ranges widely across the study area. According to Wangda and Ohsawa (2006), the annual mean temperature decreases linearly with elevation from 18.2°C to 4.3°C at the ridge top (3550 m). Mean annual precipitation (1999–2004) ranges from 882.6 mm at Lobesa (1450 m) through 1032.6 mm at Lumitsawa (2180 m) and to 1575.5 mm at Dochula (3185 m). The study area has six vegetation types: chirpine forest (CP), cool broad-leaved forest (CBL), warm broadleaved forest (WBL), mixed conifer forest (MC), meadows (MD), and agriculture land (AGR) based on the classification by Bhutan Land Cover Assessment 2010 (LCMP, 2010), Wangda and Ohsawa (2006), and Grierson and Long (1983).

Study design

The Modified-Whittaker Plot (MWP) (Stohlgren *et al*., 1995) is used to assess the graminoid diversity in different habitat types. A comparative vegetation sampling in a disturbed mixed-grass prairie by Lies and Engle (2015) showed that the Modified-Whittaker plot provides higher species richness per unit effort than contiguous quadrat methods. Moreover, nested quadrats of increasing size have been recommended to quantify species-area curves (Mueller-Dumbois and Ellenberg, 1974). The MWP design was developed to be applicable for multiple habitat types and to minimize the statistical problems of the original Whittaker plot while generating higher species richness of the Long Thin Plot (Stohlgren *et al*., 1995).

Sample sites for MWP were selected with the help of ARC GIS™, LCMP (2010), and Google Earth™ using stratified random sampling. The stratified vegetation includes Chirpine forest, Warm broad-leaved forest, Cool broad-leaved forest, Mixed conifer forest, Meadows, and Agriculture land. Each vegetation type was stratified separately using 1 x 1 km^2 grids (Figure 1). Within each vegetation type, four MWPs were placed (Stolhgren *et*

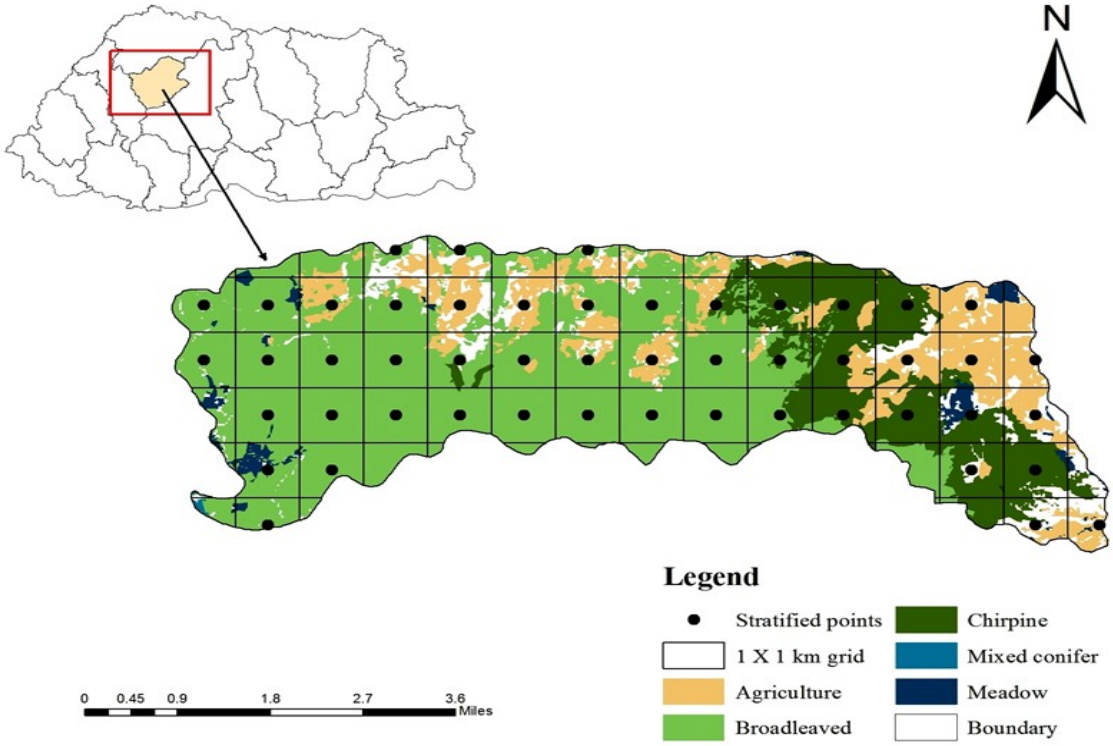

Figure 1: Map of the study area showing different habitat types with 1 x 1 km^2 grid stratification

al., 1995). The MWP measured 20 x 50 m (1,000 m^2), with 10 sub plots of 0.5 x 2 m (1 m^2) arranged systematically inside and adjacent to the plot perimeter, two 2 x 5 m (10 m^2) subplots in opposite corners, and a 5 x 20 m (100 m^2) subplot in the centre (Figure 2). The frequencies of plants were measured in the 1 m^2 subplots. Cumulative plant species within the two 10 m^2 subplots, 100 m^2 subplot, and 1,000 m^2 were recorded to account for the total alpha richness. Fieldwork was conducted from December, 2016 to February, 2017. At each site, variables such as altitude, aspect, slope, crown coverage among others were also recorded. Mean annual temperature, annual precipitations were computed following the method used by Dorji *et al.* (2015). Evapotranspiration and water balance were calculated following the method employed by Dorji *et al.* (2016). Specimen identification was carried out at the National Herbarium, Serbithang using Flora of Bhutan (Noltie, 2000; 1994) and the Grasses of Burma, Ceylon, India and Pakistan (Bor, 1960). A Nikon stereomicroscope was used to examine minute flower details. Voucher specimens were deposited at the National Herbarium in Serbithang.

Data analyses

The preliminary data were processed using pivot-table in Microsoft Excel 2010. The 10 nested 1 m^2 plots were clubbed to account for the total alpha richness and diversity, and dominance. Species from the 1,000 m^2 plot was used to compute species accumulation curve using EstimateS ver. 9.1.0 (Colwell, 2012). Species diversity was measured using Shannon's diversity, H' = ΣPiLnPi; where *Pi* is the proportion (*n/N*) of individuals of one particular species found (*n*) divided by the total number of individuals found (*N*), *Ln* is the natural log, and *Σ* is the sum of the calculations. Species richness was calculated using the formula (S_R) = (S-1)/ Log N; where *S* is the sum of species, *N* is the total number of all species.

Using Statistical Package for Social Science (SPSS), One-way ANOVA was applied to test the significance of differences between diversity indices and environmental variables in different vegetation types following Taft *et al*. (2011).

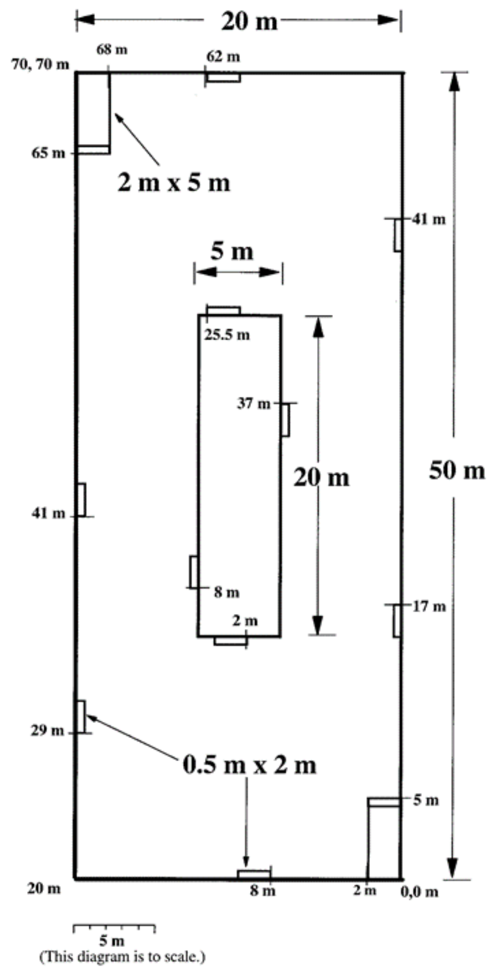

Figure 2: Plan for Modified-Whittaker Plot (Stohlgren *et al.*, 1997)

Using PC-ORD version 5.1 (McCune *et al.*, 2002,), cluster analysis was performed using distance measure of Relative Sorensen (Bray-Curtis method) and Group Average as linkage method (Tobgay, 2013). We used flexible sorting of 0.50 for grouping. Cluster analysis was used to divide the quadrats into groups based on the similarities of species or environmental characters (Chahouki, 2013). Qualitative similarity among sample groups was determined using Sørensen's index (Mueller-Dombois and Ellenberg, 1974).

Nonmetric Multidimensional Scaling (NMS) was carried out to examine variance in species composition among sites in addition to the computation of correlations between the ordination and selected environmental and structural variables following Taft *et al.* (2011). Environmental variables such as elevation, crown coverage, aspect, slope, mean annual temperature, and relative humidity were combined with vegetation parameters like Shannon index, richness, dominance, and number of graminoid species. NMS was conducted on autopilot at 400 randomizations using Sorensen distance measure with Monte Carlo test to determine ordination stress. Pearson's correlation (*r*) was used to see the strength of correlation of variables between axes. Venn diagram for species overlap between different vegetation types was made using biovenn (http://www.biovenn.nl/).

Results and Discussion

Species composition and dominance

A total of 268 plant species from 79 families were recorded from 24 Modified-Whittaker Plot set up in six vegetation types (Annexure 1), of which 235 plant species were identified. Overall, 110 graminoid species were recorded from the study area. Poaceae was represented by 45 genera, Cyperaceae by 12 and Juncaceae by only 1 genus. Of the 79 families recorded, 31.81% (21) families were represented by single genus and 19.7% (13) families were represented by two species. In the Modified Whittaker plots, herbs and shrubs were the most dominant plant group comprising 48% (n = 129), followed by graminoids at 33% (n = 90), trees 13% (n = 30), and pteridophytes (n = 12). Of the total species, 108 plant species had only single occurrence, while 64 had only 2 occurrences.

Species diversity patterns in different vegetation types

Species richness (S_R) and Shannon-Wiener Index were compared between vegetation types (Table 1). Mean alpha richness ranged from 16–38 species per MWP between vegetation types. Similarly, Shannon index ranged from 1.91–2.81. One-way ANOVA showed significant difference in species diversity $F(5,18) = 3.289$, $p = .028$, and richness $F(5,18) = 3.940$, $p = .014$ with the highest Shannon diversity in Agriculture ($H' = 2.81$). The lowest Shannon index was observed in Meadow (*H'* = 1.65).

The present study suggests that Agriculture land is more diverse than other vegetation types (Table 1). This result is in conformity to Loos *et al.* (2014), who reported agriculture land being more diverse than forest but differs in being less diverse than grasslands. In contrast, Wagner *et al.* (2000) found meadows to be more diverse at the alpha level but less diverse at the gamma level. They attribute these findings to the higher habitat heterogeneity within sites for agriculture landscapes. This, however, is not true for the current study. Even when compared to two broad-leaved forest types, species richness and diversity for Meadow (*H'* = 1.56, S_R= 4.53) suffer in comparison to the Agriculture (*H'* = 2.81, S_R= 11.21).

The composite plot is generated from the cumulative 1 m^2 plot from the 24 Modified Whittaker Plots established in different forest types (Figure 3). The most dominant family was Poaceae, found in all forest types followed by Cyperaceae. Juncaceae was found only in Agriculture landscape. No single graminoid species was present in more than five vegetation types. *Agrostis micrantha, Yushania microphylla, Poa annua,* and *Arthraxon hispidus* were found in four vegetation types. Of this, Agriculture had the highest number of unique species (*n* = 24) followed by Chirpine forest (*n* = 11).

Table 1: Summary of species diversity in different vegetation types

Vegetation type	Alpha diversity*	Gamma diversity*	Shannon-Wiener	Species richness
Chirpine	25	81	1.91	8.51
Agriculture	38	94	2.81	11.21
Warm broad-leaved	29	94	2.27	8.6
Cool broad-leaved	25	80	2.19	8.54
Mixed conifer	23	65	2.32	7.49
Meadow	16	61	1.56	4.53

*Alpha diversity = number of species in one MWP (1000 m^2); Gamma diversity = number of species in vegetation type or four MWPs (4000 m^2)

The highest percentage of graminoid was found in Chirpine forest (86%) followed by Meadow (84%). The graminoid composition in Chirpine forest was primarily dominated by Poaceae (98%, *n* = 29). This may be due to the dry climate and a lower relative humidity, which is characteristic of this vegetation type. Miller (1987) has reported occurance of *Cymbopogon* grassland dominated dry areas between 700–2100 m. The association of *Cymbopogon* spp., *Apluda mutica*, and *Heteropogon contortus* to Chirpine forest is reported by Noltie (2000) and Miller (1987), which are often burnt by forest fire.

Agriculture, despite showing a relatively lower percentage of graminoids (73%), had the highest overall graminoid species richness (*n* = 49). This vegetation is represented by 36 species of Poaceae, 10 species of Cyperaceae and 1 from Juncaceae. These findings are partially in agreement if not complimentary with that of Parker (2000) on the important weeds of rice in the warm temperate agroecological zone. This current study has also recorded the occurance of additional 15 graminoid species in addition to the book.

The Warm broad-leaved forest had the lowest percentage of graminoid species (25%, *n* = 16). This may be because of a higher percentage of herbs, shrubs, and pteridophytes. Dorji (2016) found higher fern diversity in the wet broad-leaved forest, which had higher canopy coverage and had marshy areas. A similar conclusion was made by Hemp (2001) who found the wetter southern belt of Mt. Kilimanjaro in East Africa harbored richer fern diversity. In one of the

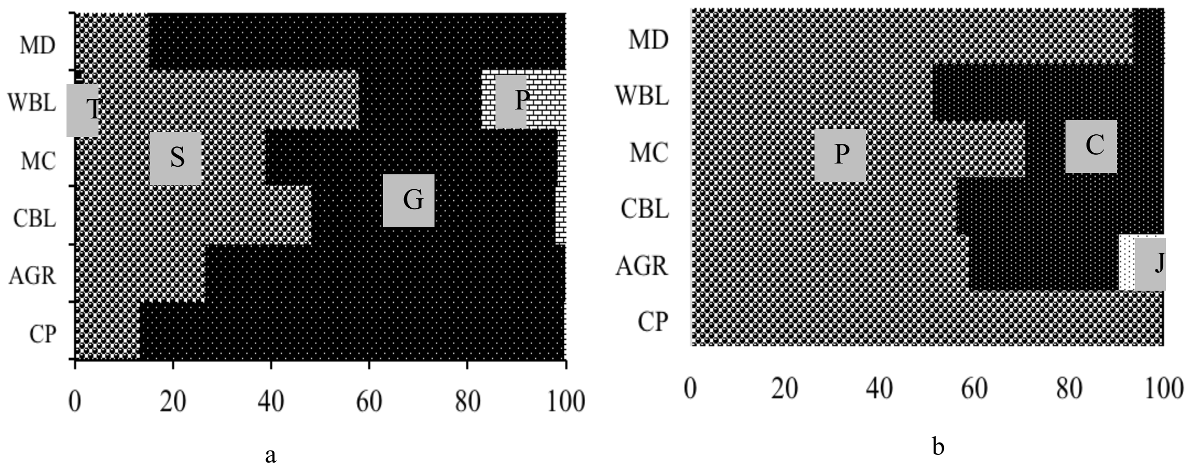

Figure 3: Percentage of a) other plant groups (T = Tree; S = Shrubs and herbs; G = Graminoids; P = Pteridophytes) and b) graminoid families (P = Poaceae; C = Cyperaceae; J = Juncaceae) in different vegetation types

MWPs, heavily dominated by *Ageratina adenophora* (56%), not a single graminoid species was recorded. Although a higher composition of Cyperaceae was recorded in the Warm broad-leaved forest (49%) and Cool broad-leaved forest (44%), these were represented by only 3 and 5 species respectively from a single genus, Carex. However, the most diverse habitat in terms of diversity was Agriculture with 14 species of sedges (7 genera).

Overall, Juncaceae was the least diverse family in the study area. Only five species were recorded from the entire study area, with only two records from the MWP. *Juncus prismatocarpus* was one of the dominant species in Agriculture fields, while *Juncus* sp. was recorded at a higher elevation in the mixed conifer forest. Other *Juncus* species such as *Juncus inflexus, J.* cf. *concinnus*, and *J. ochraceus* were found in Cool broad-leaved forest and mixed conifer forest. Records from Flora of Bhutan (Noltie, 2000) indicate that the family is mostly associated with higher elevations.

This study supports the hypothesis that graminoid diversity changes with different vegetation types. While the classic model includes only the number of species richness, it is important to account the species itself to see species overlap. However, it must be noted that in ecosystem dynamics, change in species is successive and not abrupt. Therefore, species overlap must be a strong consideration, and future studies must address this along with other environmental factors.

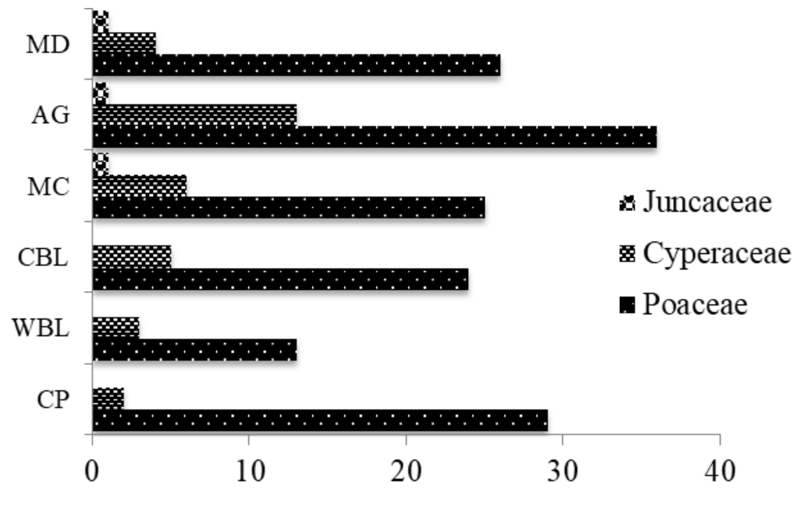

Figure 4: Number of species of Poaceae, Cyperaceae, and Juncaceae in different vegetation types

Classification of grassland types

A cluster analysis for the six vegetation types was carried out using species variance with 50% information remaining based on Relative Abundance generated from the cumulative 1 m^2 nested plots (Figure 5). Three broad groups of vegetaitons were identified based on cluster dendrogram. Chirpine forest and Chirpine meadow formed the first group, while Agriculture formed the second group. Cool broad-leaved, mixed conifer, Warm broad-leaved forest, and Broad-leaved meadow formed the third group.

The three different types of grasslands were identified through cluster analysis using Sorensen Similarity Method. Type I falls in the Chirpine zone where the air is dry and mean annual temperature is 20.65ºC. The dominant species are *Arthraxon lancifolius, A. hispidus, Heteropogon contortus, Cymbopogon pendulus, Capillipedium assimile, C. parviflorum, Schyzachrium delavayi, Themeda triandra* var. *laxa, Arundinella bengalensis, Digitaria ciliaris, Cymbopogon khasianus, Oplismenus burmanii, Sporobolus fertilis, S.*

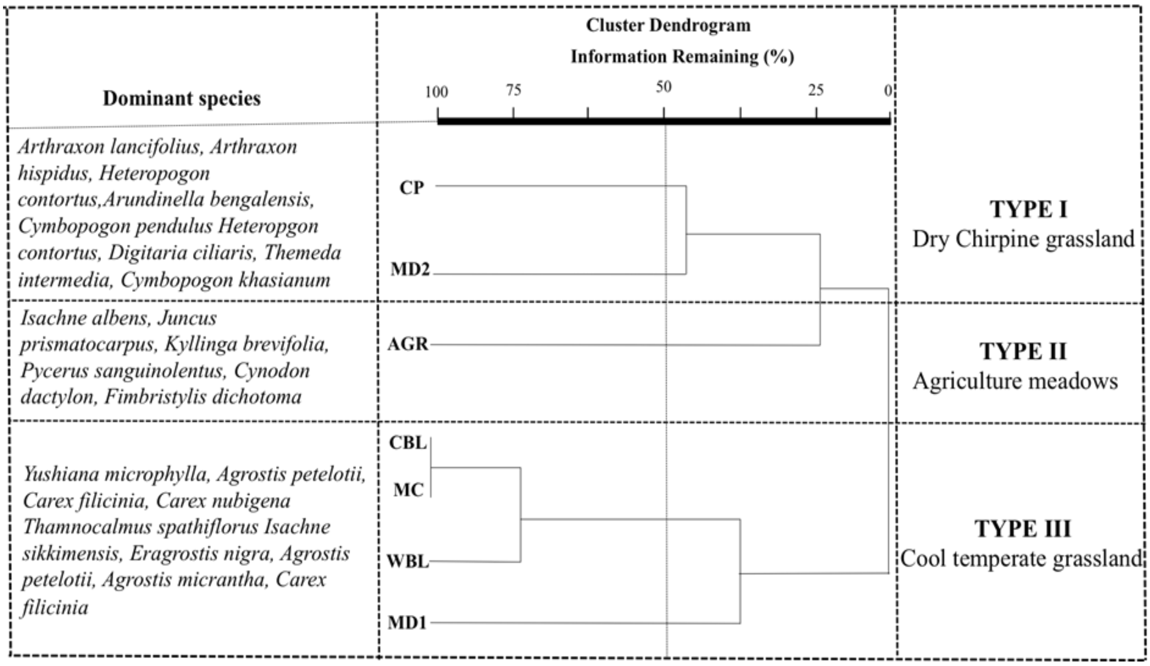

Figure 5: Cluster dendrogram showing three grassland communities

diander, Sacciolepsis indica, Fimbristylis complanata, Apluda mutica, Cyperus cyperoides, Agrostis micrantha, *Eragrostis nigra,* and *Imperata cylindrica.* Other indicator species include *Indigofera dosua, Oxalis cuneata, Galium aparine, Duhaldea cappa,* and *Bidens pilosa.* Miller (1987) also reported these species to be characteristic of dry sites, forming subtropical grasslands associated with Chirpine forests.

Type II (Agriculture meadows) occurs mostly in cultivated land and flooded rice fields with mean annual temperature of 15ºC. Agriculture land had forty-nine graminoid species, of which 53% (n = 26) were unique to this habitat type. This includes 15 species from Poaceae, 9 from Cyperaceae and one from Juncaceae, altogether represented by 24 genera. Some of the most dominant species are *Isachne sikkimensis, Juncus prismatocarpus, Kyllinga brevifolia, Pycerus sanguinolentus, Cynodon dactylon, Setaria pumila, Fimbristylis dichotoma, Bothriochloa bladhii, Capillipedium assimile, Paspalum distichum,* and *Arthraxon quartinianus.*

Type III (Cool temperate grassland) was

dominated by bamboos, *Agrostis* spp., and *Carex* spp. and had lower temperature (6.38ºC) and higher humidity (85.8%). The area had sparse tree cover compared to Type I. The transition from the dry Chirpine grassland to Cool temperate grassland is highly corroborated with the transition of mixed broad-leaved forest from Chirpine forest (Wangda and Ohsawa, 2006). They found that the transition occurs with increasing soil moisture content. In the current study, this is correlated with increasing humidity and decreasing evapotranspiration (3). The dominant plant species include *Yushania microphylla, Carex filicinia, C. nubigena, Agrostis petelotii, A. micrantha, A. brachiata, Isachne sikkimensis, Poa annua, Thamnocalamus spathiflorus* var. *spathiflorus, Oplesmenus compositus* var. *rarariflorus,* and *Drepanostachyum intermedium.* Other dominant associates are *Fragaria nubicola, Hemiphragma heterophylla, Lycopodium* sp., *Pteris critica, Ainsliaea aptera,* and *Ageratina adenophora*.

There is a gradual transition of graminoid composition between forest types. The *Cymbopogon* spp., *Arthraxon* spp., and *Heteropogon contortus* are replaced with *Yushania microphylla, Agrostis* spp., and *Carex* spp. with increasing elevation. According to Stapleton (1994b), *Y. microphylla* has hollow rhizomes, which may allow it to succeed on flatter and wetter sites. Similarly, *Carex* prefers cold and moist habitats, reaching its greatest diversity in the Kashmir Himalaya and represents one of the largest genera in higher altitude (Haq *et al.,* 2011).

Meadows were classified based on their occurances on forest types through cluster analysis with similarity index at 50%. Therefore, as a test of Beta diversity, Sorensen index was calculated to see the shared species between other forest types. Sorensen similarity was 54% between Cool broad-leaved forest and Broad-leaved forest meadows. Also, 62.5% of the total graminoid species in the Broad-leaved forest is shared with Cool broad-leaved forest. Similar results were obtained when Meadows were compared to Warm broad-leaved forest (Sorensen index of 51% and 56% shared species). Further, dominant species such as *Yushania microphylla, Agrostis petelotii, A. micrantha* and *Carex filicinia* appear to occur intermittently throughout these vegetation types which demerit a separate classification. From the 16 species known in the Broad-leaved meadows, 56% (*n* = 9) were present in Cool broad-leaved forest and

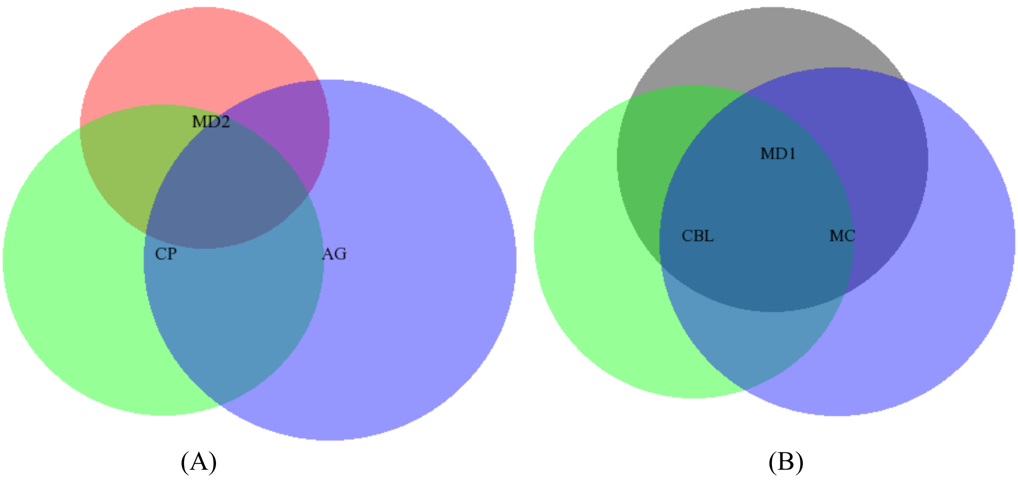

Figure 6: Venn diagram of species overlap between a) CP, AG and MD12 and b) CBL, MC and MD1. CP = Chirpine, AG = Agriculture, MD2 = Chirpine meadow; MD1 = Broad-leaved meadow; CBL= Cool broad-leaved forest; MC = Mixed conifer. All circles in Figure A and B are proportionate to the number of species within each group. However, A is not proportionate to B.

62.5% (n = 10) were shared with the mixed conifer forest. Similarly, seven of the total 15 graminoid species recorded in the Warm broad-leaved forest were common to the Cool broad-leaved forest (Figure 6).

At the habitat scale, there was considerably more overlap in species composition between Chirpine forest and Chirpine meadow. Combined species lists of each habitat type showed that 60.6% of the graminoid species are shared between these two habitat types. The dominant species in both the vegetation types was *Heteropogon contortus* which accounted with an average of 33% of the total Relative Abundance of the habitat type. Sorensen dissimilarity index was higher in Agriculture with other vegetation types. There were 20 unique species in the Agriculture. Moreover, some of the dominant species of Agriculture such as *Juncus prismatocarpus, Pycerus sanguinolentus,* and *Arthraxon quartinianus* were recorded only from this vegetation type. The third grassland type was classified as the Cool temperate grassland. Although the Cool temperate meadow was clustered in a separate subgroup, there was 82% graminoid species overlap with the other three vegetation types.

Relation between grassland communities and environmental factors

Results from NMS indicated that a three-dimension solution provided the best configuration (stress value 11.03, $p < 0.01$, Monte Carlo randomization test). Coefficients of determination for the correlations between ordination distances cumulated to r = .673 in the three-dimension. Axis 1 was correlated with water balance and precipitation, separating agriculture meadows from the rest. Axis 2 had association with higher temperature and evapotranspiration, separating Chirpine grassland. Axis III was correlated with higher elevation, relative humidity, crown cover and slope, and separates the cool temperate grassland from the other (Figure 7).

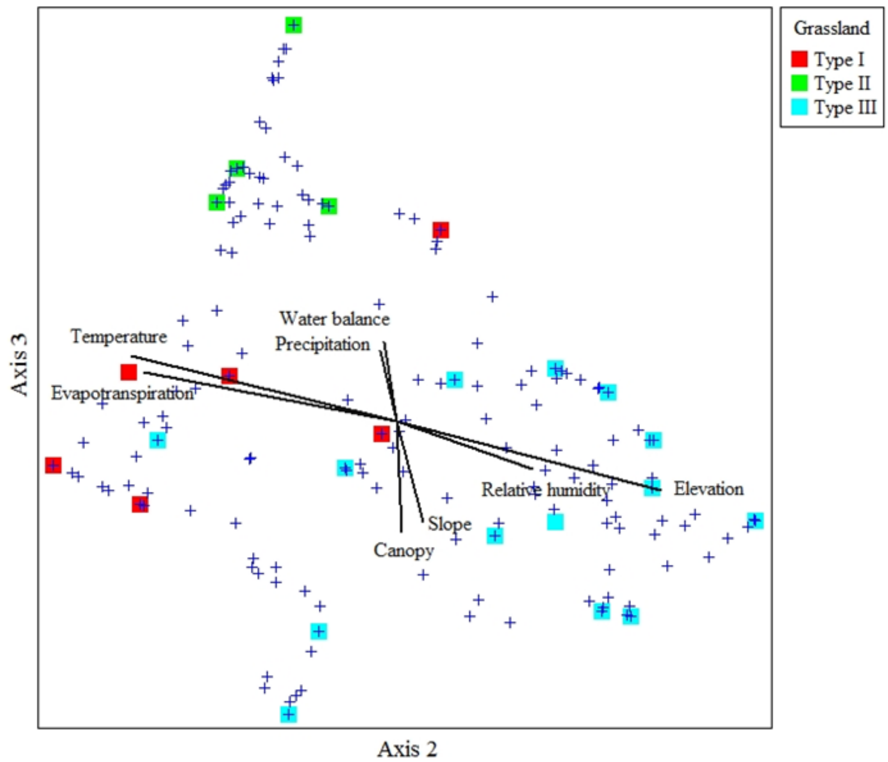

Figure 7: Nonmetric multidimensional scaling ordination diagrams showing A) Axes 1 (r^2 = .369) and 2 (r^2 = .206) and B Axes 1 and 3 (r^2 = .118).

The NMS biplot showed a clear pattern of species distribution with various grouping of environmental factors. In general, Type I showed high correlation with dry climate patterns such as higher temperature and evapotranspiration. Mean annual temperature was significantly higher in Type I. Type II showed a higher correlation with lower crown coverage, water balance, and mean annual precipitation. Species of drier habitats such as *Cymbopogon pendulus, Arthraxon lancifolius,* and *Arthraxon hispidus* formed a distinct grouping around this zone. This zone is independent of altitude and humidity in the current study area as the plots fell in different elevation zones and forest types. The transition from Zone 1 to Zone 3 occurs from the Warm broad-leaved forest from 2200–3000 m, which correlates with increasing humidity and elevation.

One-way ANOVA showed a significant difference in altitude, crown coverage, aspect and disturbance in different vegetation types ($p < 0.01$). Mean values for vegetation and environmental parameters for each vegetation class are shown in Table 2. Pearson Correlation showed that there is a positive correlation between species diversity with altitude and aspect, crown and slope. However, species richness showed a negative correlation with altitude and aspect.

Bhutan's geographic feature has attributed to a great variation in climatic condition even across relatively small areas (Dorji *et al.*, 2015). Wangda and Ohsawa (2006) reported a decreasing mean annual temperature and increasing volumetric soil moisture content with elevation, and an altitudinal difference of more than 2000 m has has rendered various forest types in the area. Changes in environmental factors such as topography, climatic conditions and disturbances can greatly influence vegetation pattern over space and time (Alexander and Millington, 2000). Therefore, species diversity is an important element to study changes in community dynamics (Hawkins and Diniz, 2004).

The transition from dry Chirpine grassland to cool humid temperate grassland along elevation gradient is consistent with the finding of Wangda and Ohsawa (2006). However, the mixed broad-leaved forest and mixed conifer forest both yielded similar grassland communities. There is a consistent association of a dry Chirpine grassland with drier sites (Miller, 1987) and *Carex* dominance in more humid sites (Haq *et al*., 2011). Similar NMS ordination result was reported by Taft *et al.* (2011), who reported that arid grassland correlates with higher temperature and decreasing elevation.

Conclusion

The study was conducted to determine the grassland communities and graminoid diversity pattern among different vegetation types on the eastern facing mountain slope of Dochula. Species diversity and richness were significantly different between vegetation types. Mean species richness between MWP ranged from 16–38. Overall, Agriculture was the most species-rich habitat ($n = 90$), while meadows had the lowest ($n = 61$). Three distinct grassland communities were identified through cluster analysis. Type I (Chirpine grassland) correlated with higher temperature and dry climate. The dominant species were *Arthraxon* spp*., Cymbopogon* spp*., Capillipedium assimile, Arundinella bengalensis,* and *Themeda* spp. Type II (Agriculture meadow) occurred in and around rice fields and fallow lands occurring intermittently between Type I and Type III, which is distinguished by the dominance of *Isachne sikkimensis, Juncus prismatocarpus, Kyllinga brevifolia, Pycerus sanguinolentus,* and *Cynodon dactylon.* Transition to Type III (Cool temperate grassland) occurs from 2000 m, which correlates with higher elevation and humidity. The dominant species are *Yushania microphylla, Agrostis* spp., and *Carex* spp. The study was conducted during the winter season, and the information collected over one season may not represent the complete population composition. Further, lack of floral characters impeded the

Table 2: Pearson's correlation efficient between biotic and abiotic factors with nonmetric multidimensional scaling axis scores

Variable	Ordination Axes		
	Axis 1	Axis 2	Axis 3
Elevation	0.78	-0.339	0.698
Slope	0.312	-0.525	0.278
Mean annual temperature	-0.781	0.325	-0.696
Precipitation	-0.19	0.419	-0.254
Evapotranspiation	-0.763	0.266	-0.668
Water balance	-0.204	0.464	-0.225
Crown coverage	-0.028	0.299	-0.194
Relative humidity	0.248	-0.594	0.14
Canopy	0.814	-0.381	0.369
Shannon index (H')	0.233	-0.078	-0.121
Evapotranspiation	0.282	-0.012	-0.017
Species richness	0.063	0.001	-0.35
Dominance	-0.318	-0.026	0.071
Total species	0.25	0.087	-0.275
Number of graminoids	-0.172	0.272	0.333
Cumulative % explained	0.31	0.44	0.67

determination of species, which may have influenced in some of the species count. Therefore, subsequent sampling spanned across different seasons is necessary to fully substantiate the overall population structure. While the study was largely successful in encompassing graminoid species within the Modified Whittaker Plots, it fails in accounting many graminoid species that occur commonly along roadsides. To address this, a combination of two or more sampling techniques is recommended for future studies. Our study is a preliminary attempt to classify grassland communities in Bhutan, which suggests that the current method can be upscaled to classify on a national level. However, additional environmental factors such as soil moisture, soil temperature, and nutrient content are required to better explain species distribution and interactions.

Acknowledgement

We would like to thank Dr. Henry J. Noltie, Dr. Chris Stapleton, and Mr. Ngawang Gyeltshen for assisting in the taxonomic identification. We thank Dr. Tom Stohlgren for his unwavering support. Our thanks to Lhendup Dorji, Dago Dorji, Lha Tshering, Karma Tenzin, Sonam Dorji, Karma Choden, Sonam Yangden, Choni Dorji, Karma Lhundup, Ugyen Tenzin, and Sherab Jamtsho for their assistance during the fieldwork. We also thank Nima Gyeltshen and Kezang Tobgay for assisting in manuscript preparation. Our sincere gratitude goes to The Explorers Club, US and the National Biodiversity Center, Serbithang for funding the research. Our special thanks to Ms. Jamyang Choden. We also thank the anonymous reviewers.

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Species name	CP	AG	CBL	MC	WBL	MD
Arthraxon lancifolius (Trinius) Hochstetter	25.46					
Arthraxon hispidus (Thunb.) Makino	16.24	1.51	1.22			
Heteropogon contortus (L.) Beauv. ex Roem. & Schult.	12.02					20.73
Grass sp. 1	11.26	0.28			0.51	
Arundinella bengalensis (Sprengel) Druce	5.88	0.58				0.31
Cymbopogon pendulus (Nees ex Steudel) Will	3.22					
Bothriochloa bladhii (Retz.) S.T. Blake	2.75	4.42				
Indigofera dosua D.Don	2.03				0.15	
Schyzachrium delavayi (Hackel) Bor	1.62				0.92	
Oxalis cuneata Jacq	1.43	5.38	0.84			0.51
Themeda triandra var. *laxa* (Andersson) Noltie	1.39	0.12				
Artemisia sp.	1.36	0.38	1.62	1.01	1.36	0.48
Capillipedium assimile (Steud.) A. Camus	1.33	2.64				
Galium aparine L.	1.26		2.28		0.04	
Desmodium sp. 1	1.26				2.24	
Oplesmenus undulatifolius var. *japonicus* (Steud.) Koidz.	1.26				1.72	
Capplipedium parviflorum (R. Brown) Stapf	1.25					
Sporobolus fertilis (Steud.) Clayton	0.94	0.28				
Lamiaceae 1	0.81					
Gerbera piloselloides (L.) Cass.	0.79					
Duhaldea cappa (Buch.-Ham. ex D.Don) Pruski & Anderb.	0.73				0.04	0.58
Hedychium sp.	0.66				0.07	0.05
Bidens pilosa L.	0.57	2.06				0.25
Ageratum conyzoides L.	0.55	2.54			0.11	
Sacciolepis indica (L.) Chase	0.52	0.46				
Chrysopogon serrulatus Trin.	0.36					
Acanthaceae 1	0.32					
Fern sp. 3	0.26	0.07	0.05			
Fimbristylis complanata (Retzius) Link	0.26					
Stellaria sp.	0.21	3.60	5.78			
Jasminium grandiflorum L.	0.19					
Spiraea sp.	0.19				0.07	
Apluda mutica L.	0.16					
Setaria pumila (Poir.) Roem. & Schult	0.16	6.26				
Berberis asiatica Roxb. ex DC.	0.11				0.44	
Cyperus cyperoides (L.) Kuntze	0.11					
Unknown sp. 8	0.11					
Unknown sp. 11	0.11					
Flemingia macrophylla (Willd.) Merr.	0.10					

Species name	CP	AG	CBL	MC	WBL	MD
Lyonia ovalifolia (Wall.) Drude.	0.08				0.33	
Plantago sp.	0.08	0.60	0.45	0.32	0.55	
Fern sp. 4	0.08					
Digitaria ciliaris (Retzius) Koeler	0.05	1.09				7.08
Pteridium sp.	0.05		0.24	0.03	0.95	0.02
Viola sp.	0.05			2.01		
Barleria cristata L.	0.03		0.03	0.09		
Berberis sp.	0.03			0.55		
Chromolaena odorata (L.) King and Robinson	0.03					
Digitaria abludens (Roem. & Schult.) Veldk.	0.03					
Digitaria longiflora (Retzius) Persoon	0.03		0.42			
Galinsoga ciliata (Rafin.) Blake	0.03					
Unknown sp. 7	0.03					
Asparagus racemosus Willdenow	0.02				0.04	
Bolbitis sp.	0.02					
Echinochloa colona (Linn.) Link	0.02	0.21				
Mimosa pudica L.	0.02					
Senecio sp.	0.02		0.60			0.08
Unknown sp. 6	0.02					
Isachne albens Trin.		8.48				
Juncus prismatocarpus R. Brown		6.94				
Kyllinga brevifolia Rottb.		6.93				
Pycreus sanguinolentus (Vahl) Nees ex C. B. Clarke		6.40				
Cynodon dactylon (L.) Pers.		6.32				
Fimbristylis dichotoma (L.) Vahl.		5.72				
Paspalum distichum L.		2.61				
Centella asiatica L.		2.46				
Equisetum diffusum D. Don		2.25			0.40	
Arthraxon quartinianus (A. Rich.) Nash		1.72				
Chrysopogon gryllus Trin.		1.71				
Fragaria nubicola (Hook.f.) Lindl. ex Lacaita		1.51	29.18	9.62	2.97	4.13
Cyperus iria L.		1.21				
Mentha spicata L.		1.19				
Digitaria sanguinalis (L.) Scop.		1.12				
Fimbristylis littoralis Gaudichaud		1.05				
Schoenoplectus juncoides (Roxb.) Palla		0.81				
Ageratina adenophora (Spreng.) King & Robinson		0.70			13.71	
Paspalum scrobiculatum L.		0.62				
Persicaria sp.		0.60				
Rumex nepalensis Spreng.		0.58	0.11			0.17
Paspalum dilatatum Poir.		0.54				

Species name	CP	AG	CBL	MC	WBL	MD
Conyza sp.		0.51				
Chenopodium album L.		0.44				
Eragrostis ferruginea (Thunb.) P. Beauv.		0.42				
Themeda intermedia (Hack.) Bor		0.40				1.68
Galinsoga parviflora Cavanilles		0.37	12.75			
Polypogon fugax Ness ex Steud.		0.31				
Echinochloa crus-galli (L.) P. Beauv.		0.30				
Houttuynia cordata Thunb.		0.25				
Eleocharis congesta D. Don		0.24				
Pleurospermum sp.		0.24				
Cyperus pilosus var. *obliquus* (Nees) C.B.Clarke		0.22				
Alopecurus aequalis Sobol.		0.21				
Crassocephalum crepidioides (Benth.) S. Moore		0.21				
Cyperus difformis Forssk		0.19				
Elsholtzia sp.		0.17	0.44			0.80
Iris sp.		0.17				
Oplismenus burmannii (Retz.) P. Beauv.		0.17				
Oryza sativa L.		0.17				
Agrostis micrantha Steud.		0.16	1.85	0.72		4.31
Xanthium indicum Roxb.		0.15				
Persicaria nepalensis (Meisn.) H. Gross		0.13				
Cirsium sp.		0.11				
Cirsium falconeri (Hook.f.) Petr.		0.08				
Coix aquatica J.Koenig ex Roxb.		0.06				
Eragrostis nigra Nees ex Steudel		0.06	0.37			9.53
Unknown sp. 9		0.06				
Potentilla sp.		0.04				
Setaria palmifolia (J. König) Stapf		0.04			0.33	
Anaphalis sp. 1		0.03				
Eleusine coracana (L.) Gaertn.		0.02				
Unknown sp. 10		0.02				
Colocassia sp.		0.01			0.04	
Eleusine indica (L.) Gaertn.		0.01				
Grass sp. 2		0.01				
Mazus delavayi Bonati		0.01				
Rhynchospora rugosa var. *griffithii* (Boeckeler) Verma & Chandra)		0.01				
Rubus ellipticus Sm.		0.01				
Carex nubigena D. Don ex Tilloch & Taylor			6.81	4.28		1.48
Geranium sp.			4.32			
Hemiphragma heterophylla Wall.			3.90	1.29		2.65
Androsace geraniifolia Watt.			3.60	5.80	0.22	

Species name	CP	AG	CBL	MC	WBL	MD
Galinsoga sp.			3.15		2.09	
Fragaria sp.			2.38	9.22		3.18
Agrostis petelotii (Hitchc.) Noltie			1.94	5.14		6.44
Poa sp.			1.56	0.78	0.84	0.32
Clinopodium sp.			1.55	0.52		0.12
Poa annua L.			1.52		0.81	
Carex filicina Nees			1.31	5.00	3.41	2.05
Oplismenus compositus var. *rarariflorus* (C. Presl.) U.			1.18		2.49	
Hydrocotyle nepalensis Hooker			1.03	0.98		
Carex munda Boott			0.88			
Selaginella sp.			0.82		15.00	
Yushania microphylla (Munro) R.B.Majumdar			0.73	21.80	0.70	0.87
Senecio chrysanthemoides DC. non Dumortier			0.60			
Geranium nepalense Sweet			0.58	2.96		
Isachne sikkimensis Bor			0.38			17.13
Diplazium sp.			0.34		0.77	0.05
Trifolium repens L.			0.34			
Microstegium nudum (Trinius) A. Camus			0.31			1.43
Hedera nepalensis K. Koch			0.24		1.14	0.02
Ilex dipyrena Wall.			0.23			
Lycopodium japonicum Thunberg			0.20	1.21		0.17
Daphne bholua Buch.-Ham. ex D. Don			0.19	1.87		
Viola biflora L.			0.18		0.33	
Swertia sp.			0.15			
Anaphalis sp.			0.12	1.78		0.15
Fern sp. 2			0.11			
Agrostis brachiata Munro ex Hook.f.			0.10	3.71		1.15
Eurya acuminata DC.			0.10			
Onychium sp.			0.10			
Ophiopogon wallichianus (Kunth) Hook. f			0.10			0.44
Pteris sp.			0.10			
Unknown sp. 4			0.10			
Gentiana bryoides Burkill			0.08			
Unknown sp. 1			0.08		0.66	
Ainsliaea aptera DC.			0.07			
Leucostegia sp.			0.05			
Carex sp. 2			0.04	1.12		
Primula denticulata Sm.			0.04			0.03
Carex baccans Nees			0.03		2.79	
Pteris cretica L.			0.03		0.04	
Centella sp.			0.01		0.33	
Cinnamomum sp.			0.01			

Species name	CP	AG	CBL	MC	WBL	MD
Fern sp. 1			0.01		0.55	
Prunella vulgaris L.			0.01			
Rubus sp.			0.01		0.11	
Gaultheria sp.				2.76		
Galium sp.				2.67		
Thamnocalamus spathiflorus subsp. *spathiflorus* Munro				2.21		0.12
Ainsliaea latifolia (D.Don) Sch.Bip.				1.58	0.73	
Carex nervosa Desf.				1.35		1.39
Carex inclinis Boott ex C.B.Clarke				1.32		0.37
Halenia elliptica D. Don				0.98	0.04	3.80
Primula gracilipes Craib				0.95		
Carex condensata Nees				0.86		
Carex longipes D. Don ex Tilloch & Taylor				0.57		
Rhododendron barbatum Wall. ex G. Don				0.52		
Festuca sp.				0.49		
Gentiana sp.				0.49		
Fern sp. 6				0.26		
Brachypodium sylvaticum (Hudson) P. Beauv.				0.23		0.46
Abies densa Griff.				0.14		
Carex remota subsp. *roechburnii* (Franchet & Savatier) Kukenthal				0.14		
Fern sp. 5				0.14		
Rhododendron arboreum var. *arboreum* (C. B. Clarke) Ridley				0.14		
Aster sp.				0.09		
Rhododendron kesangiae D.G. Long & Rushforth				0.09		
Elymus sikkimensis (Melderis) Melderis				0.09		
Agrostis sp.				0.06		
Asteraceae 2				0.03		
Calamagrostis emodensis Griseb.				0.03		
Rosa sp.				0.03		
Piper sp.					18.12	
Carex sp. 1					4.58	
Unknown sp. 3					3.04	
Parochetus communis D. Don.					2.38	
Drepanostachyum intermedium (Munro) Keng f.					1.98	
Dicliptera sp.					1.87	
Labiatae 2					1.25	
Clematis sp.					0.88	
Viburnum erubescens Wall.					0.59	
Rubia cordifolia L.					0.51	
Eulalia quadrinervis (Hack.) Kuntze					0.48	
Drepanostachyum khasianum (Munro) Keng f.					0.48	

Species name	CP	AG	CBL	MC	WBL	MD
Murraya sp.					0.44	
Unknown sp. 2					0.44	
Disocorea sp.					0.37	
Desmodium sp 5					0.29	
Dichroa febrifuga Loureiro					0.29	
Urtica dioica L.					0.26	
Desmodium sp. 3					0.18	
Desmodium sp. 2					0.15	
Girardinia diversifolia (Link) Friis					0.15	
Rhododendron sp.					0.15	
Unknown sp. 5					0.15	
Bohemeria sp.					0.11	
Desmodium sp. *4*					0.11	
Dracocephalum sp.					0.11	
Quercus griffithii Hook.f. & Thomson ex Miq.					0.11	
Wrightia sp.					0.11	
Ardisia macrocarpa Wall.					0.07	
Nepeta lamiopsis Benth. ex Hook.f.					0.07	
Rubia sp.					0.07	
Schefflera sp.					0.07	
Goodyera sp.					0.07	
Thalictrum sp.					0.04	
Cymbopogon khasianus (Munro ex Hackel) Stapf ex Bor						1.61
Asteraceae 1						1.36
Sporobolus diander (Retz.) P. Beauv.						1.07
Dactyloctenium aegypticum (L.) Beauv.						0.46
Rhodiola sp.						0.46
Imperata cylindrica (L.) Raeusch.						0.44
Cassia occidentalis L.						0.03
Carex sp.						0.02
Juncus sp.						0.02
Total	**100**	**100**	**100**	**100**	**100**	**100**