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Master's Thesis

Regeneration of dominant tree species in a temperate broadleaf forest of Bhutan Himalayas with special reference to grazing and fencing

ブータンヒマラヤの温帯広葉樹林における優占樹種の更新と放牧の影響

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1. Introduction

1.1. Background

The Himalaya is the world's highest mountain range which stretches from northern Pakistan, India and Nepal to Bhutan and is a home to more than three billion people that are very closely related with these mountains spiritually, ecologically and economically. The vast variations in climatic, altitudinal and edaphic factors has resulted in a very rich and unique floral and faunal diversity that these mountain forests were termed as the Himalayan hotspot. Forests in these mountains form a very important natural resource base for sustenance of livelihood, conservation of biodiversity and lately as a carbon sink in mitigating climate change.

The temperate broadleaf forest dominated by oaks (*Quercus* spp.) is a very widely distributed forest type along the Himalayan range between 2000 to 3500 m above sea level (Singh & Singh, 1992). Principally, forest dominated by brown oak, *Quercus semecarpifolia* Sm. are of high significance due to their strong connection with the farming systems in the temperate regions (Shrestha, 2003; Gajendra & Singh, 2012). The farmers practice subsistence farming where they raise agricultural crops with livestock and depend on the brown oak forests for fuel wood, fodder and manure. Brown oak is viewed as the farmer's best friend and a tree of multipurpose use in the temperate region. The evergreen leaves serve as an important fodder resource during the dry winters when all the grasses dry up while the strength, durability and permanence of the wood found its use as fire wood, agricultural implements, producing charcoal and sericulture (Singh et al., 1997).

Throughout the Himalayan range old-growth oak trees dominate the canopy of these forests and they support the vast range of plant and animal species to survive under the canopy and are known as the "keystone species" without which the whole ecological balance would collapse (Shrestha, 2003). The species is regarded as a late successional species forming pure stand in undisturbed climax forest. However, forests dominated by brown oak are seldom seen as pure stands but mostly occur mixed with conifers indicating frequent human disturbances.

Oak forests are also indispensable for the protection of watersheds as these forests are strongly correlated with the production of quantity and quality mountain springs (Singh & Singh, 1986; Singh & Pande, 1989; Valdia, 1998; Shrestha, 2003; Singh & Rawat, 2012).

However, in the last one century rapid population growth and economic development in the Himalayan countries have put severe pressures on the forest ecosystem particularly the brown oak forests (Singh & Singh, 1986). This may result in irreversible loss of some important species from these forests and thus are of high conservation significance. Human activities such as logging and forest road construction have damaged the oak trees while rapid increase in the cattle population overgrazed the forests destroying the young seedlings. The young leaves of brown oak are highly preferred by grazing animals. Seedlings and saplings in the natural forests are overgrazed by wild animals and large herds of sedentary and migratory cattle. Both the biological diversity as well as the livelihood sustenance of farming system in this region is threatened due to degradation of oak forests. For example, Sherestha & Paudel (1996) reported that severe degradation of brown oak in some parts of Nepal has made farmers difficult in livestock farming, due to fodder scarcity and noticeable decline of wild dove and barking deer populations which eat and live on the acorns of this oak (Sherestha & Paudel, 1996).

The widespread environmental and habitat degradation of the Himalayas are well described by Ives and Messerli (1989), known as the theory of Himalayan degradation in their book titled "The Himalayan Dilemma: Reconciling Development and Conservation". More than 70% of natural vegetation has been reported to be lost as a consequence of human activities (Singh & Rawat, 2012). In order to prevent such environmental degradation, it is important to understand the extent how harmful human activities can impose on our natural environment, and certain measures should be adopted timely to prevent the loss of important plant and animal species. The failure of brown oak (*Quercus semecarpifolia*) to regenerate in the Himalayan region is one such problems which may have severe impact on the biodiversity. Shrestha & Paudel (1996) highlighted that the Himalayan oak forests are under severe threat and need urgent concern for the mountain environment. Singh et al, (1997) described it as a "likely candidate" for "environmental semi-surprises" that has the potential to become a serious issue if not dealt at early stage (p. 371). Broadleaf forests in the Himalayas harbor the remainder of a climax flora and fauna and are important from the conservation point of view. Studying and understanding the dynamics of the lifecycle of tree species and anthropogenic activities that possibly threaten these tree species are very important as well as interesting too.

There are many factors that threaten the brown oak populations such as overexploitation of resources through overgrazing, excessive lopping of young trees for livestock fodder and frequent forest fires which destroy the regeneration and survival of recruits (Vetaas, 1999). In the Himalayas, the oaks are distributed at a higher elevation close to the alpine meadows which are traditionally the grazing grounds of migratory herds of livestock (Singh et al., 1997). This traditional practice is common to all nations in the Himalayan region where the forest dominated by brown oak are severely grazed and browsed by migratory sheep and goat in India and Nepal. Bhutan is not an exception. The temperate broadleaf forests are entirely grazed by herds of migratory yaks and cattle throughout the year.

1.2. Forest grazing in broadleaf forests of Bhutan

The kingdom of Bhutan is one of the few countries situated entirely on the Eastern Himalayas. The country presents a very diverse geography with very deep valleys and high mountains. The rugged mountains and varied climatic conditions have resulted in a very rich biological diversity that only few countries in the world could match with Bhutan. Unlike most of the countries in the world, Bhutan's commitment to conservation of forests and the rich biological diversity it harbors is very clear from the constitution which states that "a minimum of sixty percent of Bhutan's total land shall be maintained under forest cover for all time" (Constitution of the kingdom of Bhutan, 2008, p. 11). The strong commitment has currently resulted in about 72 % of Bhutan's total area under forest cover, out of which broadleaf forests occupy about 34.4 % of the total land cover in Bhutan (LUUP, 1994) and is a vital resource for the Himalayas (Sargent et al., 1985).

The temperate broadleaf forest dominated by Oaks (*Quercus semecarpifolia*) is distributed at an elevation of 2400-3200m asl and is found often mixed with conifers and other temperate broadleaf species. The temperate broadleaf forest forms one of the most valuable forest types of Bhutan and are very important from the conservation point of view because they deliver many ecosystem services like conservation of soil, water and also act as a "lifeline" for many native plant and animal species (Singh & Rawat, 2012, p. 29).

Forest grazing is widely practiced in the forests of Bhutan, and is intermingled with the sustenance and livelihood of Bhutanese people (Norbu, 2002). The whole forest in Bhutan is commonly grazed by domestic animals, migratory herds of cattle and wild animals. Forest provides the richest source of fodder and broadleaf forests are widely preferred for grazing due to the diverse range of fodder options. About 90% of Bhutanese households own cattle which freely graze in the forests and meet up about 22% of the total national fodder requirement from the forests (Norbu, 2000; Roder et al., 2002). The leaf litter collected from the forests which are converted to compost and manure plays an important part in maintaining agricultural soil fertility (Roder, et al., 2003). In the temperate region where the choice of tree species is limited, meeting fodder requirement during the dry winter months is the greatest challenges farmers face in sustaining their livestock similar to other Himalayan nations. Most of the tree species are deciduous in nature and remain leafless in winters. Evergreen species like brown oak in the temperate region are therefore highly valuable fodder species to the farmers (Roder, 1992). It is during this period of time that the oak trees are severely lopped for fodder while throughout the year young oak recruits are heavily grazed and trampled by large herds of migratory cattle and yaks. Many researchers from all over the world have reported the poor and uncertainties in regeneration of oak (*Quercus* spp.) in these forests. Very limited studies have been done in Bhutan despite the fact that the regeneration of *Quercus* spp. in these forests is problematic. Like in any parts of the world, the views amongst the researchers on the beneficial or harmful effects of forest grazing thus remain divided. Several researchers in Bhutan agree that grazing has had important impact on regeneration and often related the problematic forest regeneration in harvested areas in Bhutan to cattle grazing (Norbu, 2002; Ijssel, 1990; Wangda and Ohsawa, 2005).

However, there is no quantitative information to justify the impact of grazing on regeneration of broadleaf species (Norbu, 2000). The only way to justify the positive or negative effects of cattle grazing that would be scientific and supported by quantitative data is to carry out some experimental studies by excluding grazing animals through fences as practiced in some countries; Linhart & Whelan (1980) in Coed Gorswen, North Wales; Kelly (2002) in Ireland, Nomiya et al. (2002) in Japan, Darabant et al. (2007) in Bhutan, etc. Such experiments are however, tedious, costly and long term in nature, that they are very limited in the Himalayas particularly in the broadleaf forests. Nonetheless, a similar study conducted in conifer forests of Bhutan documented the positive effects of forest grazing on conifer tree regeneration; "Forest grazing facilitates tree regeneration in a conifer forest with palatable bamboo understory" (Darabant et al., 2007). By nature and composition of species, broadleaf forests differ largely from the conifer forests and therefore findings from the conifer zone could not be applied to the broadleaf forests. Kelly (2002) and Perin et al. (2006) reported the benefits of exclosure in protecting and greatly enhancing the survival of *Quercus petraea* seedlings from grazing in a national park in southwest Ireland, while a similar study in woodlands of Coed Gorswen, North Wales failed to show any effect of exclosure on *Quercus rubur* and *Q. petraea* seedling (Linhart & Whelan, 1980).

1.3. Changes in leaf morphological traits

There are so many factors which can influence the survival of oaks. Apart from survival and increment in morphological features like height, collar diameter and age, leaf traits such as leaf surface area, leaf moisture content and leaf mass per area (LMA) are very important which can explain the growth characteristics of plants and determine whether they grow in stressful environments. Studies related to these topics in the brown oak, *Quercus semecarpifolia* is very new and would be interesting too. As plant stresses are displayed through their functional leaf traits, leaf mass and leaf surface area can be used as a tool to study the plant-stress environments (Bussotti, 2008). High LMA (leaf dry weight/surface area) values are related to climatic (rainfall, temperature and solar radiation) stresses (Ogaya and Penuelas, 2007; Wright et al., 2002; Field and Mooney, 1986). Higher values of LMA are also related to drought, and aging of plants (Bussotti, 2008). Herbivory (Eliot and David, 2002) and anthropogenic stresses such as increased troposphere ozone concentration (Bussotti, 2008) can also influence the leaf traits. LMA is primary trait in the carbon economy of plants which represents the investment required per unit leaf area (Wright et al., 2002) and is a very important survival strategy of the plants. Leaves of plants on dry and nutrient-poor soils have thick, hard leaves with thick cuticles and accordingly have higher LMA which enable them develop resistance to wilting. Besides, high LMA in leaves are seen as a part of adaptation to the environment which makes the leaves to have longer leaf life span and prepares them more tolerant to physical hazards. It is thought that changes in the leaf traits may be a part of structural reinforcement to acclimate to grazing pressures which will render the leaves more tolerant to harsh environment and browsing.

2. Research goals and objectives

The study is carried out with the general objective to understand the forest structure and role of local topographic features in the regeneration of tree seedlings. Specifically, the objective of this study is- to assess the regeneration of a temperate broadleaf forests with special emphasis on the brown oak (*Quercus semecarpifolia)* and the effect of grazing activities on their characters by comparing natural forests (hereafter referred as "unfenced") and exclosures (hereafter referred as "fenced").

In this study, we hypothesized that repeated cattle grazing may reduce the photosynthetic capacity of oak seedlings through reduction in leaf surface area and lower their survival chances. Besides repeated grazing by cattle could also have profound changes in the leaf traits (such as LMA) of this species which is currently unknown. Therefore the study also aimed to study the influence of grazing and other variables (drought, age of seedlings and soil moisture and compactness) on LMA of *Quercus semicarpifolia* seedlings growing in fenced and unfenced experimental plots.

The study is expected to increase the knowledge-base for scientific understanding of broadleaf forests in Bhutan.

3. Methods

3.1. Study area

The study was carried out in an evergreen oak forest (Sargent et al., 1985) at Chimithankha, Gidagom under Thimphu District, Bhutan (Figure 1) at an elevation of 2800-3100 m asl. The upper storey of the forest was dominated by brown oak (*Quercus semicarpifolia*) with few other species as *Tsuga dumosa* and *Abies densa*. There are also few deciduous trees of *Acer*, *Betula* and *Corylus* species mixed with other evergreens like *Ilex* and *Rhododendron* species in the middle storey. The understorey is mostly covered with bamboo (*Yushania microphylla*) and *Daphne bholua* and *Pieris formus* (Tashi, 2003). The forest is very diverse in fodder resources and is grazed throughout the year by cattle in summers and yaks in winters without giving time for the browsed seedlings to recover (Department of Forests, 1992). There are three kinds of grazing animals in the study area. Migratory cattle consist of about 56 % of the grazing animals which graze in the study area from May until October every year. The migratory cattle then moves to lower altitude to avoid the cold winters. As soon as the migratory cattle move to lower areas, the migratory yaks which form 30% of total grazers move down to the study area from higher altitude and graze for the next 6 months from November to April. The sedentary cattle owned by local people forms about 14 % of the total grazers and graze the area throughout the year (Source: Socio-economic survey, April 2011) The local people from the villages nearby also use the forest to extract fuel wood, fodder and leaf litter (Tashi, 2003).

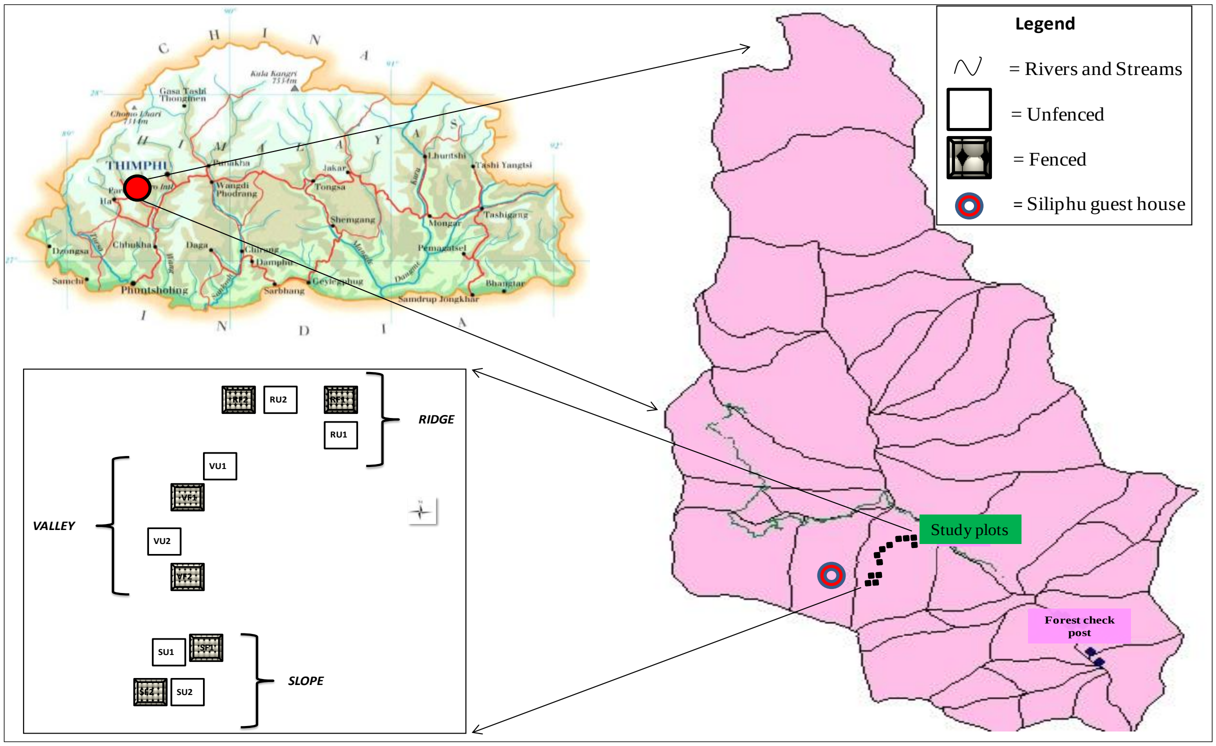

Figure 1. Map showing the location of Bhutan (left) and Bhutan map showing the location of study area and plot layout.

3.2. Climatic condition

Air temperature was recorded using HOBO data loggers enclosed in solar radiation shield (Onset Computer Co. MA, USA) mounted on a pole at 1.3 m above the ground near the Siliphu guest house (Figure 1). The climatic condition recorded from 2006 to 2011 shows a significant increase in the mean annual temperature during these years. Annual mean temperature recorded was 8^{0}C with the maximum temperature of 18^{0}C and minimum of -6^{0}C (Figure 2).

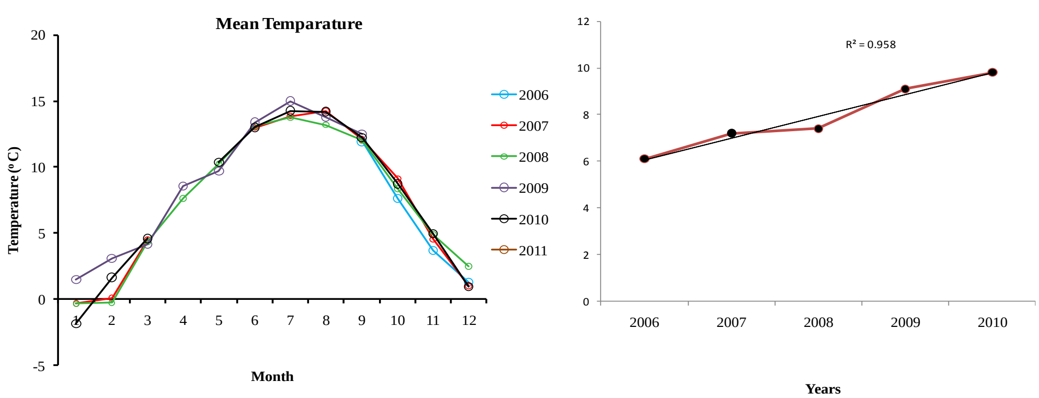

Figure 2. (left) Mean monthly temperature of different years; (right) increasing trend in the mean yearly temperature of last five years. Note: The scale of the temperature is different for the two figures. Data source: RNR RDC Yusipang.

Soil moisture content was measured by hydro-sense (CD 620 + CS 620, Campbell Scientific Inc., Logan, Utah) bearing 12 cm and 20 cm probes. The mean value of five measurements for each probe was used as the soil moisture of a plot. Soil hardness was measured by a push cone (Yamanaka's soil hardness tester, Kiya Seisakusho, Tokyo). Soil hardness value was obtained from the mean of 5 random penetrations into the soil in each plot.

3.3. Vegetation survey

Vegetation survey plots were set on three sites based on the local topography, namely, ridge, valley and slope. Vegetation survey was done in 3 categories: 1). Tree and regeneration survey, 2). Shrub layer survey 3). Herbs layer survey. Tree layer survey was carried out in 2010 in 12 circular plots of radius 15m. All tree species above 1.3 m (Breast height) were measured for their height (m) and diameter at breast height (dbh, cm).

In order to study the effects of grazing by animals on tree seedling regeneration, six pairs of fenced and unfenced plots of sizes 10 m × 10 m were established in 2000. Each fenced plot has an adjacent comparable unfenced plot. To avoid path worn by cattle and edge effects of fenced plots, unfenced plots were placed at least 3-5 m apart from the fence.

Seedling regeneration and establishment were measured by counting all the tree seedlings in the 10 m × 10 m plots. Every seedling was recorded with their collar diameter (mm), height (cm), intensity of grazing, age and micro-site on which they grow. Age of seedlings was estimated by counting the branch tiers or bud scale scars (Wangda, 2006). The regeneration of tree species was grouped into seedlings (Height less than 25 cm) and saplings (Height taller than 25 cm but DBH less than 10 cm). The term "recruits" was used to refer to the total of seedlings and saplings.

For shrub layer, every shrub species within each plot were recorded with their cover (%) and natural height (cm) of the tallest individual.

In the herb layer survey, 5 quadrats of 1 m × 1 m were set for each 10 m × 10 m plots (4 quadrats at corners and one at the center). Cover (%) of each species and the height of the tallest individual of each species were recorded.
To estimate the canopy structure and light condition on the forest floor, 4-5 hemispherical photos were taken from the center of each 10 m × 10 m plots at 50 cm above the ground.

Identification and nomenclature of trees, shrubs and herbs were followed to: "Flowers of the Himalaya" (Polunin & Stainton, 1984), "Flowers of the Himalaya: A supplement"(Stainton, 1988), "Flora of Bhutan"(Grierson & Long, 1983-2000; Noltie,1994-2000), "The Orchids of Bhutan" (Pearce & Cribb, 2002), “Weeds of Bhutan” (Parker, 1992), “The plant book” (Mabberley, 1997) and “Wild Rhododendrons of Bhutan” (Pradhan, 1998).

3.4. Leaf samples

To examine the influence of grazing on the morphological traits of seedlings, a total of 142 leaf samples were collected from 31 (13 from fenced and 18 from unfenced plots) brown oak seedlings (height< 2.5m, collar diameter < 5cm, age 3 to 12 years).

The leaf samples were carefully put in airtight polythene bags to prevent evapo-transpiration and their fresh weight was measured on the same day of collection. Weight of individual leaves were measured by a sensitive electronic balance (Mettler Toledo PM4000) towards the nearest 0.01 g. Projected area of the leaves was measured by using a flat-bed scanner and free software LIA32 for Windows (available online at http://www.agr.nagoya-u.ac.jp/~shinkan/LIA32/LIAMan.htm). Each of the leaves was weighted after oven dried at 80^0 C for 3 days. Leaf moisture content was calculated from the differences between fresh weight and dry weight of each leaf. LMA values were calculated as a ratio of leaf oven dry weight and the projected leaf area.

$$\mathrm{LMA} = \frac{\text{Oven dry weight}}{\text{Leaf area}}\ (\mathrm{gcm}^{-2})$$

Dried leaf samples were ground into powder by mill and were sent to Soil and Plant Analytical Laboratory (SPAL), Ministry of Agriculture, Thimphu, Bhutan for analysis of foliar Nitrogen (N) content.

3.5. Grazing intensity

The intensity of grazing on the individual recruits from which the leaves were collected was judged on a 1-6 scale based on the visual observations of individual plants. A scale of 0 was assigned to the seedlings and saplings that are not grazed. 5-6 scale refers to heavily grazed recruits with top/leading shoot completely grazed; 3-4 scale to recruits with top shoot present but leaves grazed; 1-2 scale refers to very lightly grazed seedlings with both leaves and top shoot which are presently in good growth form.

3.6. Socio-economic survey

To collect basic information about the study area and to understand the type of grazing system practiced, a rapid socio-economic survey based on structured questionnaires were used. All migratory herders (6 yak herders and 5 cattle herders) who graze their animals in the study area were interviewed. Further, 10 households of the village Chimithangkha, which is closest to the study forests were interviewed.

3.7. Data analyses

BA (basal area, cm^2) was calculated from dbh for each tree as cross-sectional area of the stem at breast height. RBA (relative basal area, %) was calculated for each species by dividing the sum of BA of each species by total BA of all species in a plot, and used as the abundance measure of species in a community. The dominant tree species in each plot was determined by dominant analysis (Ohsawa, 1984; Kikvidze & Ohsawa, 2002).

For the shrub and herb layers, the volume of each species was estimated by multiplying height (cm) with coverage (%). The volume estimate or relative volume was used for the analysis of dominant species in these understory layers. The mean canopy openness (%) and mean canopy closure (%) of each plot were measured by analyzing hemispherical photographs using the free computer software LIA for Win 32 (LIA32), downloaded and available on the internet at (http://hp.vector.co.jp/authors/VA008416).

Leaf area index (LAI) which is defined as the projected leaf area per unit ground area (m^2/ m^2) was calculated from the hemispherical photos using LIA32. Statistical analysis was performed by SPSS ver. 16 (SPSS Japan Inc., Tokyo, Japan).

Regeneration counts were compared between fenced and unfenced plots and between my survey in 2011 and previous data taken in 2002 (2002 data source: RNR RDC Yusipang). A repeated measure ANOVA (rmANOVA) was used to test the significance of fencing and year. One-way ANOVA was used to test the significant difference of seedlings and saplings counts between unfenced and fenced plots. Two samples Kolmogorov-Smirnov test and two samples *t*-test were used to determine the significant difference while comparing the morphological traits of recruits of unfenced and fenced plots. Linear regression was conducted to examine the relationship between LMA and other environmental variables like soil moisture content, grazing intensity, soil hardness etc. The relationship between species distribution and environmental variables were done through canonical correspondence analysis (CCA) by using the software CANOCO for windows 4.5 (Microcomputer power).

4. Results

4.1. Floristic composition of three major life forms

A total of 35 tree species were recorded from the study area which were grouped into three major life forms of evergreen broadleaf, deciduous broadleaf and evergreen conifers. Eight species belonged to evergreen broadleaf group while sixteen and three species belonged to deciduous broadleaf and evergreen conifer groups respectively. Evergreen species consisted of Fagaceae (*Quercus semecarpifolia, Q. thomsoniana),* Oleaceae *(Osmanthus suavis),* Aquifoliaceae (*Ilex dipyrena)* and Ericaceae *(Rhododendron arboretum, R. barbatum, R. griffithianum* and *R. thomsonii)* while deciduous broadleaf species consisted of Aceraceae (*Acer campbellii, A. hookeri, A.pectinatum, Acer sterculiaceum*), Betulaceae (*Betula alnoides, Corylus ferox*), Oleaceae (*Fraxinus floribunda*), Araliaceae (*Gamblea ciliata*), Rosaceae (*Pyrus insignis, Malus baccata, Prunus ceresoides, Sorbus microphylla*), Caprifoliaceae (*Viburnum ner-*

vosum) and Lauraceae (*Lindera neesiana, Litsea doshia*). Evergreen conifer consisted of families of Taxaceae (*Taxus baccata*) and Pinaceae (*Tsuga dumosa* and *Picea spinulosa*).

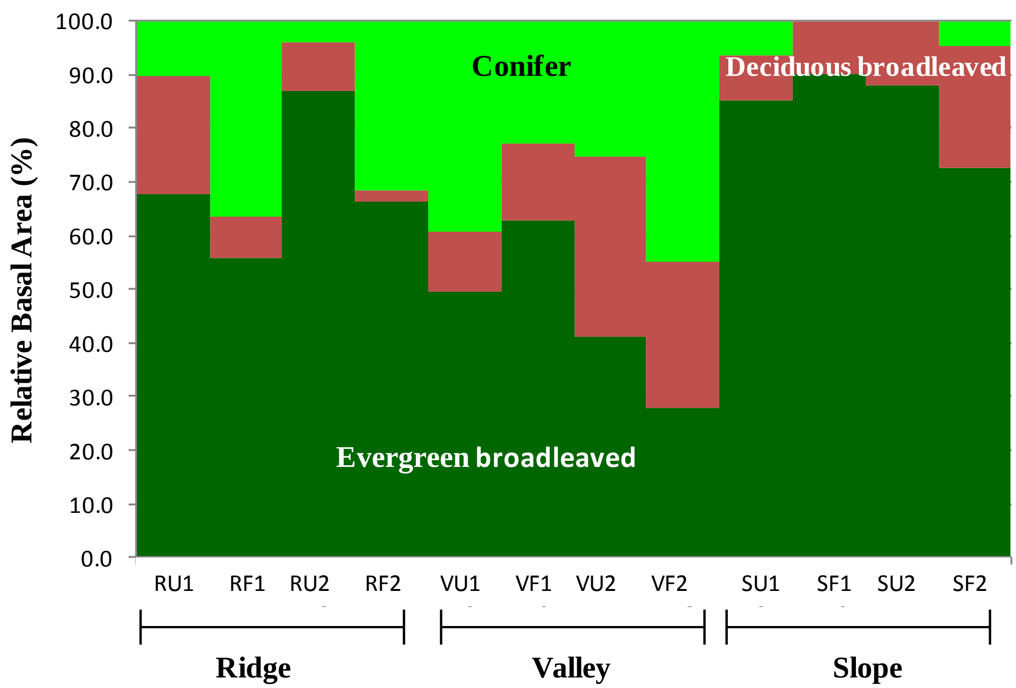

Figure 3. Abundance of trees categorized into three life forms of evergreen broadleaf, deciduous broadleaf and conifer

All the plots are dominated by evergreen broadleaf tree species (Figure 3). The average share to relative basal area of three life forms; evergreen broadleaf, deciduous broadleaf and conifers are 66.2 %, 15.1% and 18.8% respectively (Figure 3). On all the locations evergreen broadleaf has the highest percent share with *Quercus semecarpifolia* being the major contributor to total relative basal area (Tab.1). Rhododendrons were more dominant on the ridges and valleys while *Osmanthus suavis* and *Ilex dipyrena* were more on the slope. Deciduous broadleaf trees were more abundant in the valleys and ridges with percent share of 21.2 % and 16.4 % respectively compared to the slopes (7.6%). The most dominant deciduous trees were of *Betula alnoides* and *Acer campbellii*. Conifer abundance was measured higher on the slopes and valleys (27.8% and 21.2% respectively) than the plots on the ridge (5.3%). *Tsuga dumosa* was the single most dominant conifer species in the valleys and slopes (Tab.1). *Tsuga dumosa* requires considerable humidity which is being met from the moist valleys and slopes. Ridge top is mostly dominated by *Taxus baccata* and *Picea spinulosa* which require strong sunlight and drier habitats (Table 1).

Table 1

Plot description along with the structural characters and tree species abundance. BA refers to basal area (m^2); DBH, diameter at breast height; RBA, relative basal area. Odd numbered plots are unfenced and even numbered plots are fenced. Shaded numbers represents dominant species in the plot. Plot numbering: R=Ridge, V=Valley, S= Slopes, U= Unfenced, F= Fenced, each pair of fenced and unfenced plots are represented by the same number.

Plot location	**Ridge**				**Valley**				**Slope**			
Plot No.	**RU1**	**RF1**	**RU2**	**RF2**	**VU1**	**VF1**	**VU2**	**VF2**	**SU1**	**SF1**	**SU2**	**SF2**
Altitude	2974	2977	2957	2957	2953	2955	2966	2969	2976	3001	2991	2978
Aspect	N	N	N	N	NW	NW	NW	N	NW	NW	NW	NW
Slope	22%	22%	21%	21%	18%	17%	21%	21%	33%	30%	26%	28%
Tree inventory plot size(m^2)	706	706	706	706	706	706	706	706	706	706	706	706
Species/plot	20	17	20	16	16	13	15	16	21	14	18	17
Total BA (m^2/ha)	48.6	49.0	40.6	49.9	69.2	37.3	36.9	53.1	66.3	60.0	85.4	62.5
Maximum DBH (cm)	72.5	160	82.5	98	180	100.5	88	112.5	115	130	190	143
Maximum Height (m)	33	37.3	29.3	27.6	32.4	37.7	30.2	34.4	42.7	41.3	43.7	36.5
Diversity (H')	2.6	2.2	2.1	2.4	1.9	2.2	2.1	2.1	2.0	1.7	2.0	2.1
Species Evenness (J)	0.9	0.8	0.7	0.9	0.7	0.7	0.7	0.7	0.6	0.8	0.7	0.7
Regeneration plot size (m^2)	100	100	100	100	100	100	100	100	100	100	100	100
Evergreen BL (RBA, %)	RBA	RBA	RBA	RBA	RBA	RBA	RBA	RBA	RBA	RBA	RBA	RBA
Quercus semecarpifolia	24.3	69.7	51.1	22.0	79.2	3.9	24.7	53.7	37.1	39.8	74.6	19.2
Quercus thomsoniana	30.9	4.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Osmanthus suavis	5.4	3.9	3.5	3.4	1.5	3.2	1.5	1.8	1.2	0.6	0.8	30.6
Ilex dipyrena	2.6	1.4	0.1	0.6	2.3	7.5	0.0	1.6	9.8	19.4	6.8	2.9
Rhododendron arboreum	3.4	5.7	15.3	8.1	7.1	8.1	14.8	5.9	1.4	6.6	4.7	3.0
Rhododendron barbatum	1.2	0.0	2.5	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhododendron griffithianum	0.0	0.0	0.0	7.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rhododendron thomsonii	0.0	0.0	0.0	46.7	0.0	5.3	0.0	0.0	0.0	0.0	0.0	0.0
subtotal	67.8	85.1	72.6	88.0	90.1	28.0	41.1	63.0	49.4	66.4	86.9	55.8
Deciduous BL												
Acer campbellii	2.6	0.0	7.7	0.0	5.0	2.9	2.2	0.0	4.4	1.2	1.2	1.6
Acer hookeri	0.0	0.0	0.0	0.3	3.2	2.9	2.9	2.6	0.0	0.3	0.0	0.3
Acer pectinatum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.1
Acer sterculiaceum	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Betula alnoides	5.2	1.5	7.6	0.0	0.0	10.4	20.1	0.0	2.3	0.0	2.8	0.0
Corylus ferox	0.8	0.3	1.1	1.1	1.1	2.5	6.2	4.2	1.3	0.0	0.2	1.6

Table 1 *(continued)*

RBA (%)												
Fraxinus floribunda	0.0	0.0	0.0	0.0	0.0	7.2	0.0	0.0	1.0	0.0	0.0	0.0
Gamblea ciliata	8.5	2.3	3.9	7.3	0.0	0.0	1.2	4.1	0.0	0.0	2.5	0.0
Malus baccata	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0
Pyrus insignis	4.3	0.7	1.4	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Prunus cerasoides	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	1.1
Sorbus microphylla	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Viburnum nervosum	0.3	0.5	0.0	1.8	0.7	0.0	0.0	0.0	1.2	0.0	0.0	0.0
Lindera neesiana	0.0	0.0	0.9	0.0	0.0	0.0	0.9	3.4	0.0	0.0	1.3	0.0
Litsea doshia	0.0	3.3	0.2	0.2	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0
Unknown	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0
subtotal	21.8	8.6	22.9	12.0	9.9	27.3	33.5	14.2	11.4	2.0	9.1	7.7
Evergreen Conifer												
Taxus baccata	5.9	5.1	4.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tsuga dumosa	2.9	1.2	0.0	0.0	0.0	44.7	25.4	22.8	39.1	31.6	3.9	36.6
Picea spinulosa	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
subtotal	10.3	6.3	4.5	0.0	0.0	44.7	25.4	22.8	39.1	31.6	3.9	36.6
Grand total	100	100	100	100	100	100	100	100	100	100	100	100

4.2. Forest structural features

The structural characteristics of each forest are shown in Table 1. The forest in the study area was very diverse and three different layers could be distinguished. The canopy layer of 35-40 m height was dominated by mature trees of oak and hemlock. Trees of *Betula alnoides, Fraxinus* spp., *Ilex* and *Rhododendron* spp. formed the sub-tree layer (10 m - 20 m), and the shrub layer (2 m – 5 m) was mostly dominated by the bamboo (*Yushania microphylla*), *Pieris formosa*, *Daphne sureil* and *Rosa* spp.

The maximum height of the forest was 37.3 m, 53.1 m and 43.7 m on ridge, valley and slope respectively. Similarly, maximum DBH were recorded as 160 cm, 180 cm and 190 cm, respectively. Both the maximum height and maximum diameter were recorded in *Quercus semecarpifolia* trees revealing that the oak trees were highly matured. Considering the forests with trees of DBH above 1m and height of more than 25 m as old growth forests (Tang et al., 2007), one can clearly say that these forests have already become very mature and are truly old growth forests. Ridge has the highest species richness with 29 species followed by slope 27 and valley 23. Ridge also recorded higher species diversity than valley and slope (Table 2).

Table 2

Forest structural features on three different locations. The values show mean ± standard deviation.

Location	Species Richness	Shannon diversity index (H’)	No. of dominants
Ridge	15 ± 2	2.3 ± 0.2	1 - 2
Valley	10 ± 1.7	2.1 ± 0.1	2 - 3
Slope	11 ± 1.7	2.0 ± 0.2	2 - 3

4.3. Regeneration of tree seedlings

4.3.1. Abundance of trees and seedlings

In all plots except one plot in the valley, mature *Quercus semicarpifolia* trees dominated the percent share of relative basal area. *Quercus thomsoniana* trees were abundant on the ridge than in the valley and slopes. Abundances of mature tree species within 20 m radius from the center of each plot were similar in fenced and unfenced plots suggesting that seed rain which is the basis for seedling regeneration is uniformly distributed. However; the count of regenerating seedlings and saplings in both fenced and unfenced plots differed from the pattern of distribution found for the mature trees. Seedling and sapling abundance did not show a clear correlation with the abundance of mature trees (Figure 4).

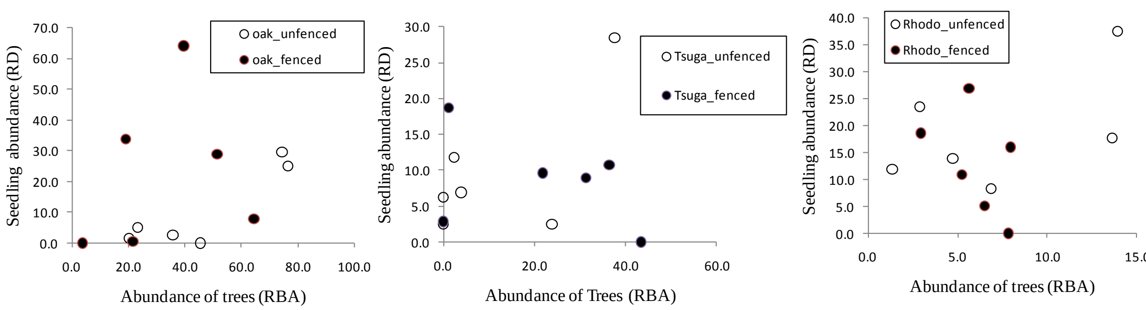

Figure 4. Relationship between abundances of mother trees (relative basal area) with seedling abundances (relative density) in each plot of size 10 m × 10 m.

There were 21 tree species regenerating in total (Table 3). There was a clear indication of seedling and sapling preferences over the local topographies. *Quercus semecarpifolia* recruits were more abundant on the slope while light demanding conifers like *Picea spinulosa, Pinus wallichiana* and *Taxus baccata* dominated in the plots on the ridge (Table 3). Deciduous broadleaf species; *Viburnum nervosum, Acer campbelli, Betula alnoides Corylus ferox* and *Litsea kingii* were more dominant on the valleys and slopes indicating their preferences on cool and moist sites.

Table 3
Relative densities of young recruits (seedlings and saplings) of tree species. Odd numbered plots represent unfenced plots and even numbered plots represent fenced plots.

	Ridge				Valley				slope			
Species	RU1	RF1	RU2	RF2	VU1	VF1	VU2	VF2	SU1	SF1	SU2	SF2
Evergreen BroadLeaved												
Quercus semecarpifolia	1.5	7.8		0.6	25.0		5.1	31.3	2.6	64.1	29.7	33.9
Quercus thomsoniana	4.4	3.1	1.3	13.1	4.2		5.1			1.3		0.6
Rhododendron arboreum	23.5	10.9	17.7	16.0	8.3		38.5	29.2	11.9	5.1	13.9	18.6
Rhododendron barbatum	1.5		1.9						0.7			
Rhododendron griffithianum				4.6								
Osmanthus suavis	5.9	6.3	0.6	1.7	2.1	12.5		2.1	0.7	3.8	1.0	
Ilex dipyrena	22.1	14.1	8.2	24.0	31.3	12.5	17.9	22.9	15.2	10.3	18.8	5.6
Ligustrum dispermum						12.5			4.6	1.3	1.0	
subtotal	58.8	42.2	29.7	60.0	70.8	37.5	66.7	85.4	35.8	85.9	64.4	58.8
Deciduous Broad leaved												
Viburnum nervosum				1.1					2.0			1.7
Acer campbelli	4.4	4.7		2.3	2.1		10.3		11.9	1.3	24.8	22.6
Betula alnoides				4.6	4.2	12.5			2.0			
Corylus ferox				0.6	2.1	12.5	2.6		3.3			0.6
Fraxinus				0.6	4.2		17.9	2.1	7.9		4.0	1.7
Gamblea		1.6										
Lindera heterophylla												0.6
Litsea kingii	2.9		8.9	5.7	6.3	37.5			6.0	2.6		3.4
Sorbus macrophylla			0.6					2.1				
subtotal	7.4	6.3	9.5	14.9	18.8	62.5	30.8	4.2	33.1	3.8	28.7	30.5
Evergreen Conifer												
Taxus baccata	4.4	7.8	23.4	2.9						1.3		
Tsuga dumosa	11.8	18.8	2.5	2.9	6.3		2.6	10.4	28.5	9.0	6.9	10.7
Picea spinulosa	4.4	9.4	13.3	7.4					2.6			
Pinus wallichiana	13.2	15.6	21.5	12.0	4.2							
subtotal	33.8	51.6	60.8	25.1	10.4	0.0	2.6	10.4	31.1	10.3	6.9	10.7
	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

Densities and relative abundances of mature trees were similar in the unfenced and fenced plots at each site (high similarity percent of over 65%) indicating that the mother trees which act as a seed source for natural regeneration is uniformly distributed (Table 4). However, there are certain exceptions with dominant species like oak (*Quercus semecarpifolia*) and some conifers which did not follow the trend. Despite the presence of large number of mature trees, the densities of seedlings and saplings of oak were much lesser than expected (Table 3). Regeneration of oak was more concentrated on the slopes than the ridges and valleys. On the other hand, more conifer seedlings especially those of *Pinus wallichiana* (18.9%), a lower elevation pine were recorded in the plots on the ridge despite the absence of mother trees in the vicinity. The seed must have been dispersed by wind from the lower *Pinus wallichiana* forests and met the favorable growing conditions due to warming climate. Seedlings and saplings of *Ilex* *dipyrena (15%), Rhododendron arboreum (15.3%)* and *Tsuga dumosa (8.5%)* formed the major portion of regeneration layer in the plots.

Table 4
Mature tree species found in the study area (data combined) and their relative abundances in each of the three sites

	Ridge		Valley		slope	
	unfenced	fenced	unfenced	fenced	unfenced	fenced
Quercus semecarpifolia	39.2	60.4	54.0	30.5	56.4	29.8
Quercus thomsoniana	15.4	2.9				
Rhododendron arboreum	9.9	9.2	11.3	7.4	3.1	4.8
Rhododendron barbatum	2.0	0.1				
Rhododendron griffithianum		4.8				
Osmanthus suavis	4.5	4.9	1.6	2.7	1.0	15.8
Ilex dipyrena	1.4	1.3	1.2	4.9	8.3	11.3
Viburnum nervosum	0.2	1.5	0.3		0.6	
Acer campbelli	5.4		3.7	1.6	2.8	1.4
Acer pectinatum						1.6
Betula alnoides	6.6	1.0	10.2	5.6	2.6	0.0
Corylus ferox	1.0	1.0	3.7	3.5	0.7	0.8
Fraxinus				3.9	0.5	
Gamblea	6.3	6.5	0.6	2.1	1.3	
Lindera heterophylla	0.5		0.5	1.8	0.6	
Litsea kingii	0.1	2.3			0.6	
Sorbus macrophylla					0.2	
Taxus baccata	5.3	3.3				
Tsuga dumosa	1.4	0.8	12.9	36.0	21.3	34.5
Picea spinulosa	0.7					
Total	100.0	100.0	100.0	100.0	100.0	100.0
Similarity %	70.0%		65.3%		65.7%	

Proportional similarity index between the relative abundances of mature trees and abundances of recruits (seedlings and saplings) were 41.5% and 38.8% respectively for unfenced and fenced plots. The total number of recruits of all tree species pooled together did not differ significantly between unfenced and fenced plots with mean count of 9417 ha^{-1} and 9167 ha^{-1} respectively (One way ANOVA, $p > 0.05$).

Table 5
Relative densities of young recruits (seedlings and saplings) recorded in the fenced and unfenced plots at each of the three sites.

	Ridge		Valley		Slopes	
	Unfenced	Fenced	Unfenced	fenced	Unfenced	Fenced
Quercus semecarpifolia	0.4	2.5	15.7	9.4	14.0	43.1
Quercus thomsoniana	2.2	10.5	4.5			0.8
Rhododendron arboreum	19.4	14.6	21.3	8.8	13.2	14.5
Rhododendron barbatum	2.2					
Rhododendron griffithianum		3.3				
Osmanthus suavis	2.2	2.9	1.1	1.3	0.8	1.2
Ilex dipyrena	12.3	21.3	24.7	7.5	17.3	7.1
Viburnum nervosum		0.8			1.2	1.2
Acer campbelli	1.3	2.9	5.6		17.7	16.1
Acer pectinatum			1.1	60.6		
Betula alnoides		3.3	2.2	0.6	1.2	
Corylus ferox		0.4	2.2	0.6	2.1	0.4
Fraxinus		0.4	10.1	0.6	6.6	1.2
Gamblea		0.4				
Lindera heterophylla						0.4
Litsea kingii	7.0	4.2	3.4	1.9	3.7	3.1
Sorbus macrophylla	0.4			0.6		
Taxus baccata	17.6	4.2				0.4
Tsuga dumosa	5.3	7.1	4.5	3.1	20.6	10.2
Picea spinulosa	10.6	7.9			1.6	
Pinus wallichiana	18.9	13.0	2.2			
Ligustrum dispermum			1.1	5.0		0.4
Total Number of species	13.0	17.0	14.0	12.0	12.0	14.0
similarity (%)	67.0%		35.9%		67.2%	

4.3.2. Seedling and sapling abundance

All seedlings with relative density less than 5 % are omitted for the sake of convenience. The seedling and sapling count of 2002, two years after fence establishment was taken as the baseline data which was compared with the current survey conducted in 2011. The mean number of tree recruits in 2002 was 1057 ha^{-1} in grazed plots (unfenced) and 1793 ha^{-1} in the un-grazed (fenced) plots. The difference in recruit densities between the two treatments; unfenced and fenced in 2002 was however not significant (one-way ANOVA, $p > 0.05$, Figure 4). In 2011, the mean densities of recruits decreased in both grazed unfenced and fenced plots with a mean count of 983 ha^{-1} and 1326 ha^{-1} respectively. The mean difference in total number of recruits between grazed and un-grazed plots in 2011 as well as the decrease in total number of recruits over the years was also not statistically significant (Paired *t*-test and rmANOVA, $p > 0.05$).

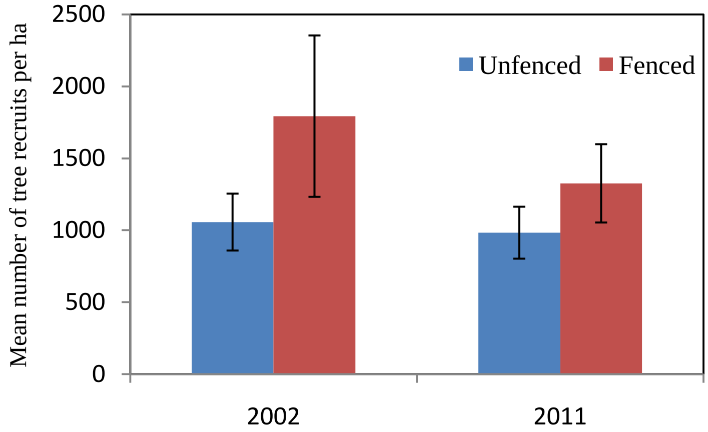

Figure 5. Mean number of recruits of tree species in unfenced and fenced plots in two measurement periods 2002 and 2011.

Analysis of data according to species revealed that the decrease in total number of recruits over the period was largely due to decrease in *Quercus semecarpifolia* recruits in both fenced and unfenced plots (Figure 6). The decrease however was not significant in both the treatments (paired sample *t*-test, p > 0.05). In the unfenced plots *Q. semecarpifolia* decreased from a mean of 966 recruits per ha to 866 recruits per hectare in 2002 to a mean of 816 recruits per hectare in 2011. The number of oak seedlings increased after fencing from 1450 per ha in 2000 to 4150 in 2002. However after 2002 there was decrease in the number of oak recruits to 2183 per ha in 2011 which could be related to the thick growth of bamboo in the fenced plots. Conifers like *Tsuga dumosa and Pinus wallichiana* seedlings and evergreen broadleaf species like *Rhododendron arboreum* and *Ilex dipyrena* counts increased largely over the period (Figure 6).

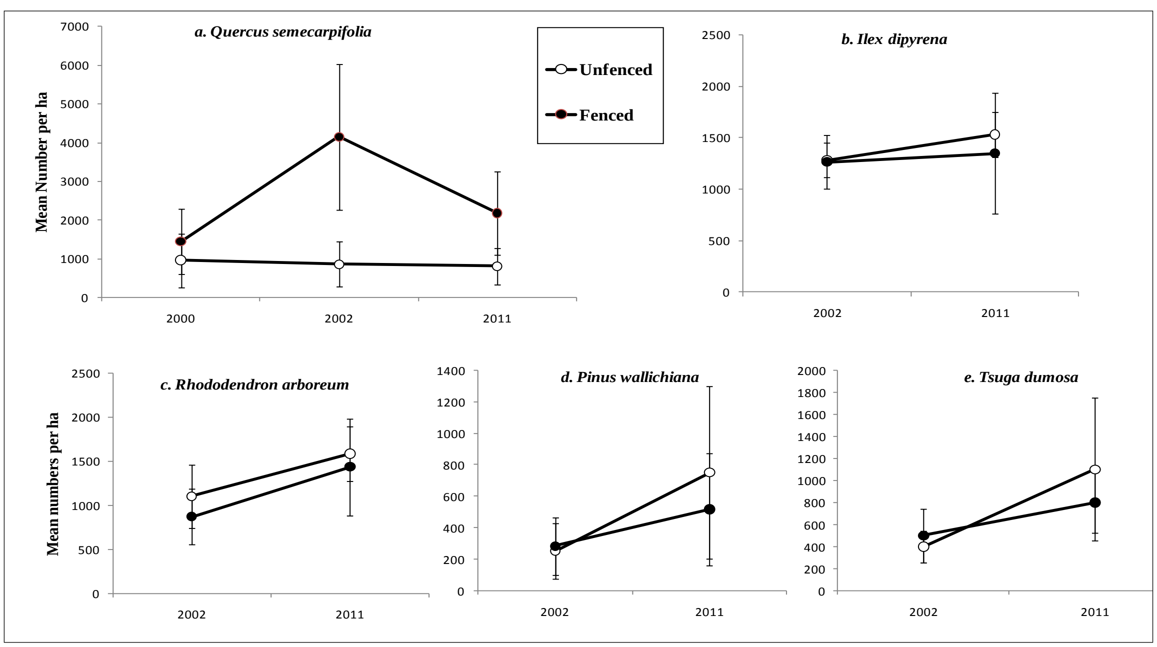

Figure 6. Mean count of recruits per ha of main tree species in fenced and unfenced plots in 2002 and 2011

Further analysis by grouping *Q. semecarpifolia* recruits into seedlings (height < 25cm) and saplings (height > 25cm) revealed that there were high mortality of seedlings in the fenced plots during the period. Large number of small seedlings (height < 10cm) of oak was recorded in unfenced and fenced plots in 2002 with only few saplings suggesting that there was a mast cropping of *Q. semecarpifolia* in the year 2000 or 2001. The survey in 2011 showed that seedlings count in fenced plots has decreased. The count of oak saplings however, has increased in the fenced plots. Fencing protected the seedlings of 2002 from grazing and permitted to grow into the sapling stage. A repeated measures ANOVA with Greenhouse-Geisser correction determined that the increase in sapling count between the two periods was significant ($F (1, 5) = 6.7$, $p < 0.05$, Table 6) with significant interaction of fencing and time (rmANOVA, $p < 0.05$). However, the decrease in seedling counts between the year 2002 and 2011 did not differ significantly (rmANOVA, $p > 0.05$, Table 4). The results indicate that fencing strongly protected the saplings from grazing animals thereby increasing their count significantly in the fenced plots.

Table 6. Repeated measures ANOVA with Greenhouse-Geisser correction for number of Quercus semecarpifolia seedlings and saplings in unfenced and fenced plots in 2002 and 2011.

Group	Time effects			Fencing effects			Time x Fencing		
	df	*F*	*p*	*df*	*F*	*p*	*df*	*F*	*P*
Seedling	1,5	3.148	0.136	1,5	2.754	0.158	1,5	3.315	0.128
Sapling	1,5	9.113	0.029	1,5	6.748	0.048	1,5	7.958	0.037

4.4. Effect of fencing on seedling and sapling abundance and composition

The mean count of recruits in fenced and unfenced plots in 2011 did not differ significantly (One-way ANOVA, $p > 0.05$). However, relative abundances of the seedlings and saplings differed between unfenced and fenced plots. The total count of seedling of *Ilex*, *Fraxinus* and all conifers (height < 25 cm) was lower in the fenced plots (Figure 7). Fencing resulted in thick growth of bamboos and brambles which could have acted as a strong competitor for light and space to the young seedlings. Species like *Q. semecarpifolia* and *Acer* seedlings were recorded more in the fenced plots than the unfenced plots. These seedlings act as shade tolerant species in the earlier stages of growth (Tashi, 2004). Grazing by animals in the unfenced plots on the other hand helped to control thick growth of bamboos and brambles and encouraged more lights to reach the forest floor thereby facilitating germination of seeds. But as seedlings grew into the sapling stage (height > 25 cm) their numbers got reduced as they become more visible and prone to browsing and trampling effects of overgrazing (Nomiya et al., 2002). Seedlings those are palatable and grazed by animals (eg. *Quercus, Osmanthus* and *Acer*) decreased in the grazed plots while the number of grazing resistant species likes *Rhododendron* sp., *Ilex dipyrena, Pinus wallichiana, and Picea spinulosa* either increased or remained constant in the grazed plots (Figure 7 & Figure 8).

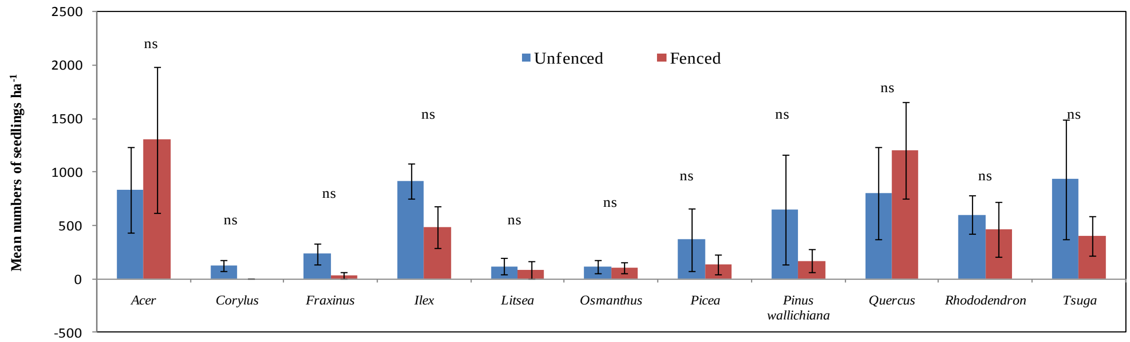

Figure 7. Mean count of seedlings per ha of main species in unfenced and fenced plots in 2011. ns refer to no significant difference (one-way ANOVA).

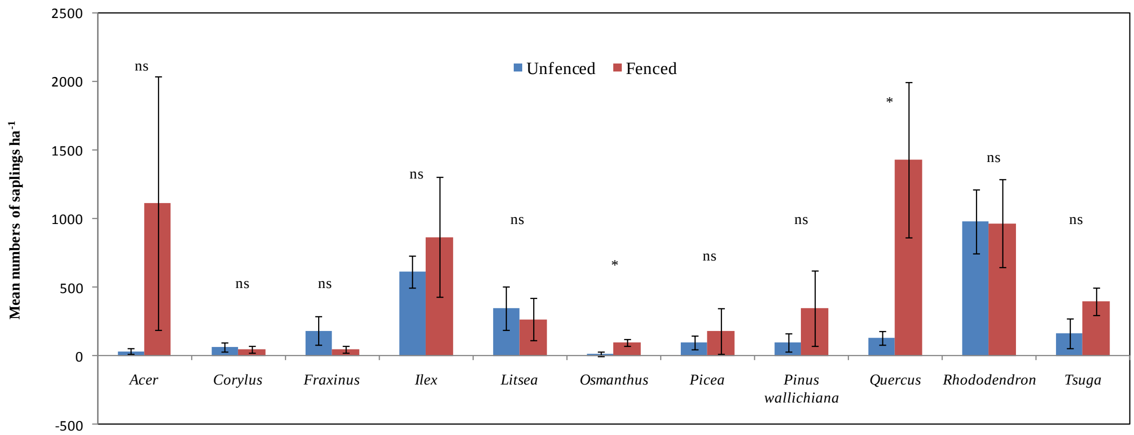

*Figure 8. Mean count of saplings per ha of main tree species in unfenced and fenced plots in 2011. Ns refers to not significant, * - significant at p=0.05, one-way ANOVA results.*

Fencing had a positive effect on the abundance of saplings (Height > 20 cm). The total number of saplings in the fenced plots was two times higher than the total number of saplings in the grazed plots (Figure 8). Sapling count of *Q. semecarpifolia* and *O. sauvis* was significantly higher in the fenced plots than unfenced plots (One-way ANOVA, $p < 0.05$, Figure 8). However, no significant differences were observed between the sapling numbers of *A. campbelli*, *C. ferox*, *F. floribunda*, *I. dipyrena*, *L. kingie*, *P. spinulosa*, *P. wallichiana*, *R. arboreum* and *T. dumosa* between the treatments (Figure 8).

4.5. Effect of fencing on morphological characteristics of oak recruits

Morphological features recruits of oak were examined in two categories: 1). Seedling's morphology that includes 3 growth parameters: height (cm), collar diameter (mm) and age (years) and 2). Leaf morphology that includes: leaf surface area (cm^2), leaf moisture content, leaf N content (N) and leaf mass per area ratio (LMA).

A very high significant difference was observed between the height distribution of total recruits of unfenced and fenced plots (two samples Kolmogorov-Smirnov test, $p < 0.001$; Figure 9a). However, there were no significant differences in collar diameter distribution between the fenced and unfenced plots (Kolmogorov-Smirnov test, $p > 0.05$; Figure 9b). In the unfenced plots there were large number of small seedlings of conifers, Ilex and Rhododendron (height < 25 cm) as compared to fenced plots (Figure 9c & 9d). Fenced plots have higher number of individuals of the upper height classes (Figure 9a). Species wise analysis showed that the significant difference between the height classes were more visible in the upper height classes with more tree saplings (height > 25 cm) especially those of oak in the fenced plots than the unfenced plots (Figure 9b).

Though there were large number of young tree seedlings in both fenced and unfenced plots (Figure 9a), the number of individuals decreased largely with increase in height classes particularly in the unfenced plots (Figure 9a). This trend was true for grazed species like *Q. semecarpifolia, A. campbellii* and *T. dumosa* while unpalatable species like rhododendron's recruitment in to upper height classes were continuous (Figure 9b and 9d).

When all recruits were pooled together, there is clear a indication that fencing is favoring the recruitment of tree species particularly in the upper height classes (Figure 9a).

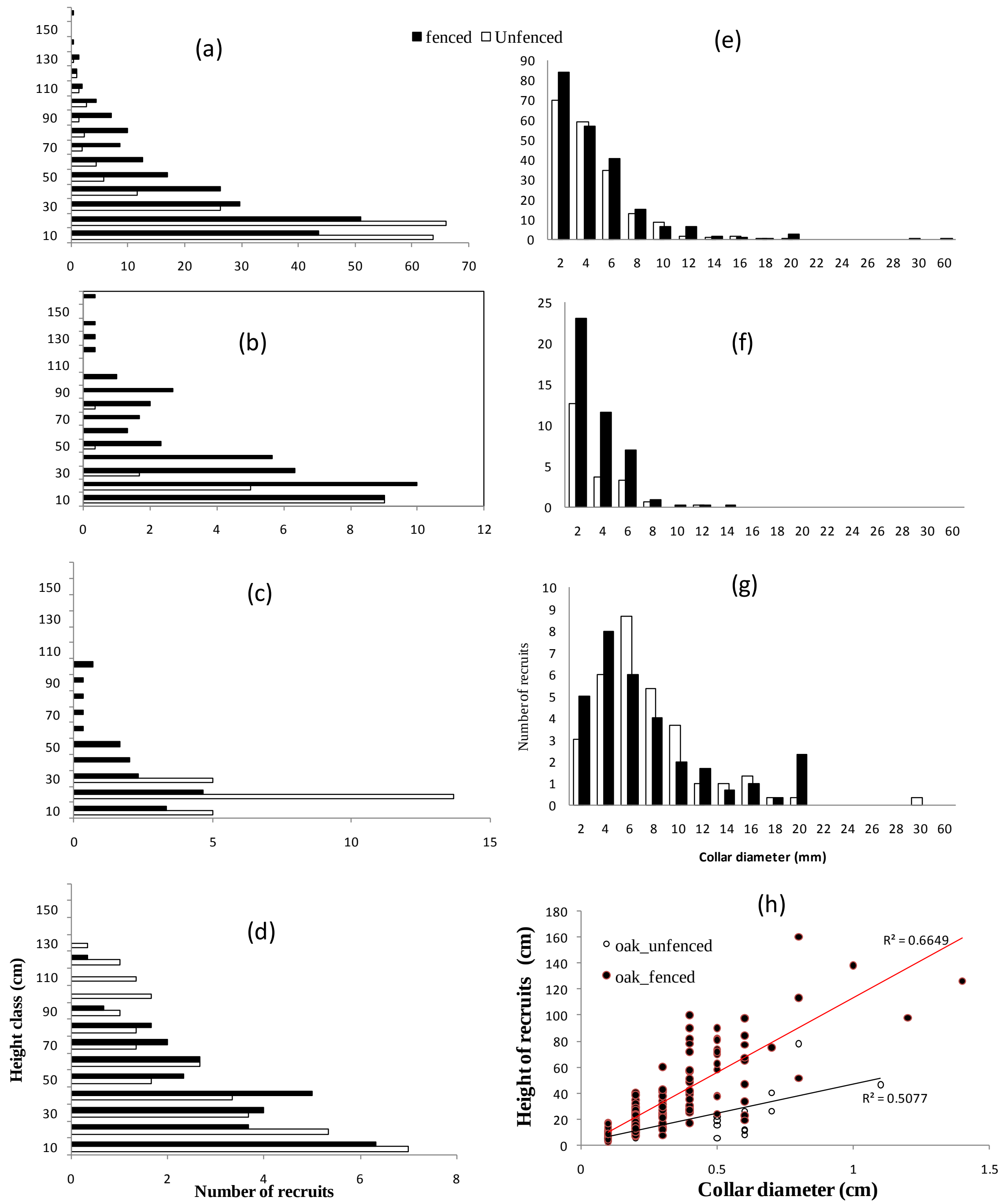

Figure 9. Height and diameter distribution of recruits of tree species: (a)-(d)Height class distribution of recruits, (a)Total recruits; all species combined, (b) Quercus semecarpifolia recruits,(c)Tsuga dumosa recruits and (d)Rhododendron arboreum recruits, (e)Diameter class distribution of total recruits, (f)diameter distribution of Q. semecarpifolia recruits, (g)Rhododendron arboreum recruits (h)Correlation between height and collar diameter of Q.semecarpifolia recruits.

4.6. Changes in height, collar diameter and age of *Quercus semecarpifolia* recruits due to fencing and grazing

The mean height of oak recruits in the fenced plots (66.1 ± 11.9) were significantly larger (t = 2.5, 29 df, two-tailed *t*-test, p = 0.02, Table 7) than the mean height of oak recruits in the grazed unfenced plots (32.8 ± 5.6). However, mean collar diameter and age of recruits did not differ significantly (two tailed t-test, p>0.05). Similar study in a conifer forest of Bhutan reported significant difference between height and collar diameter of seedlings between grazed and un-grazed plots showing more abundance of seedlings and saplings in the unfenced plots (Darabant et al., 2007). Our study was quite contradictory with this study.

Table 7
Results of t-test for differences between unfenced and fenced plots. SE refers to standard error. Bold letter represents significant difference at p =0.05.

	Unfenced	Fenced	*t*-test		
	Mean ± SE	Mean ± SE	df	t	p
Height (cm)	32.8 ± 5.5	66.1± 11.9	29.0	2.5	**0.02**
Collar diameter (mm)	7.4 ± 1.2	5.5±0.9	29.0	1.2	0.239
Age (years)	9.4 ± 0.9	10.4±1.1	29.0	0.7	0.47
Soil hardness (kg)	3.0 ± 0.4	1.92±0.2	29.0	2.4	**0.025**
Soil moisture content	23.6 ± 2.2	18.6±0.9	29.0	2.1	0.051

Simple linear regression was computed to assess the relationship between the height of oak recruits and collar diameter. There was a positive relationship between the two variables ($r^2 = 0.673$, n = 215, p < 0.001, Figure 9h). When the relation was compared separately for unfenced and fenced plots, the relationship became further stronger in the fenced plots ($r^2 = 0.67$, n = 158, p < 0.001) than the unfenced plots ($r^2 = 0.50$, n = 57, p < 0.001). A scatter plot summarizes the results (Figure 9h). Overall, there was a strong, positive correlation between height and collar diameter in the fenced plots compared to the unfenced plots revealing that repeated grazing of the top shoot in the unfenced plots has limited the height growth of the oak recruits while collar diameter remained unchanged thereby weakening the correlation between height and collar diameter in the unfenced plots.

4.7. Changes in leaf traits of *Q. semecarpifolia* recruits due to grazing and fencing

During the field survey several morphological differences were observed with leaves of seedlings and saplings of fenced and unfenced plots. As seedlings of oak were the target species as well as the mainly grazed species, leaf physical features like thickness, surface area and leaf mass per area (LMA) were measured and compared between unfenced and fenced plots. In the unfenced grazed plots about 60% of the seedlings were heavily grazed, 28 % grazed while only 2 seedlings were not grazed. Leaves from unfenced seedlings which were repeatedly grazed demonstrated significantly high thickness and significantly lower surface area as compared to the leaves of seedlings obtained from fenced plots (One-way ANOVA, $p<0.001$, Figure 10a & 10b) revealing that the leaves of grazed seedlings were smaller in size and thicker than the un-grazed leaves. The LMA (g cm^{-2}) was significantly higher for the unfenced and grazed seedling leaves than the fenced seedling leaves (One-way ANOVA, $p<0.05$, Figure 10c). The foliar N content of unfenced grazed leaves was significantly lower than the fenced leaves (One-way ANOVA, $p < 0.05$, Figure 10d)

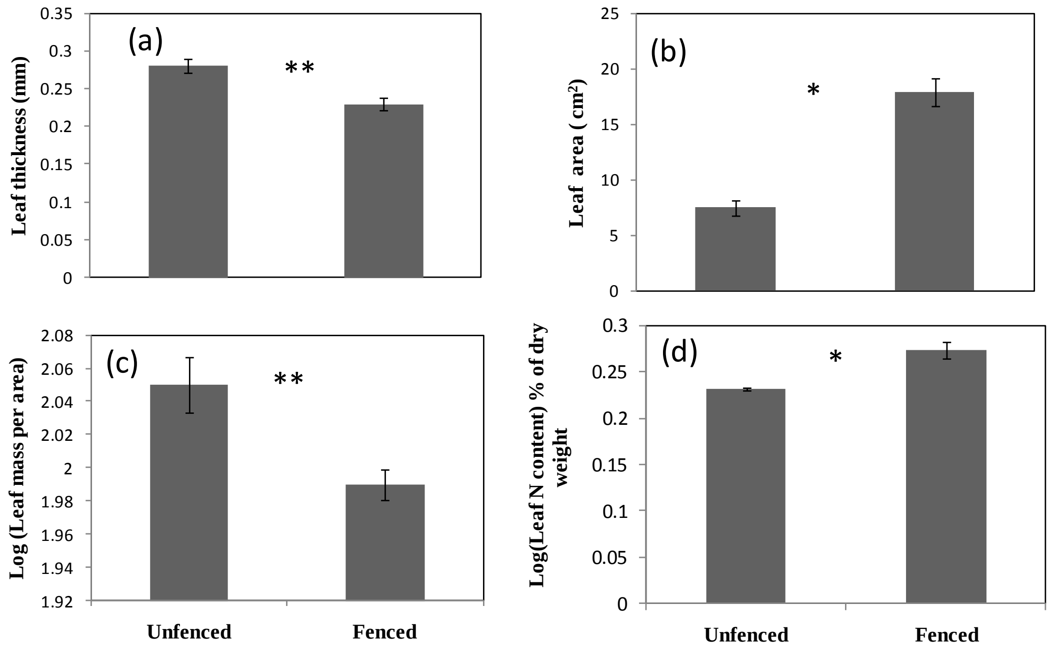

*Figure 10. Comparison of leaf traits of seedlings of unfenced and fenced plot. a)Leaf thickness b)projected leaf area c)Leaf mass per area and d)Leaf N content. Labels indicate results of one-way ANOVA tests, **P < 0.001, *P < 0.05, Bar represents standard error of the mean.*

Results of the linear regression analysis indicated that LMA was positively related to soil moisture content, soil compactness and browsing intensity. The relation between LMA and soil compactness was weak (r^2=0.042, $p > 0.05$; Figure 11a).It grew stronger with soil moisture content (r^2= 0.193, $p < 0.05$; Figure 11b) and was strongest with grazing intensity (r^2= 0.373, $p < 0.001$; Figure 11c). LMA showed a strong negative relationship with canopy cover (%) (r^2= 0.452, $p < 0.05$, Figure 11d) and accordingly it was negatively correlated with LAI (r^2= 0.429, $p < 0.05$, Figure 11e). LMA was also negatively correlated with foliar N concentration. The relationship was not very strong (r^2=0.1594, $p >0.05$; Figure 11f). Leaves of seedlings growing in fenced plots showed higher N concentrations compared to the leaves of seedlings from unfenced plots. On the other hand the LMA was higher for the leaves of seedlings growing in unfenced plots than the fenced plots. These differences have resulted in a distinct grouping of seedling leaves from fenced and unfenced plots as shown in the Figure 11f.

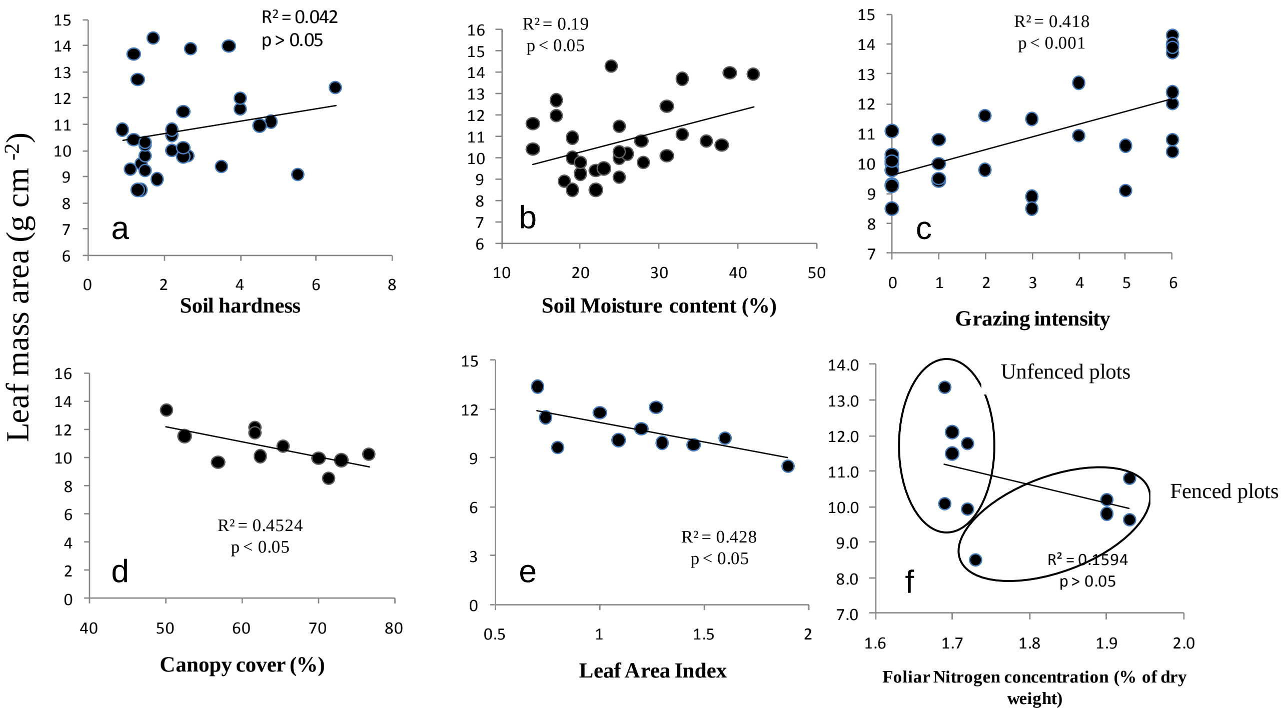

Figure 11. Linear relationship between LMA of oak leaves with (a) soil hardness, (b) soil moisture content, (c) grazing intensity (graded on 1-6 scale: 5-6=heavily grazed (top shoot completely missing and leaves grazed), 3-4= Grazed (top shoot present but leaves grazed, 1-2 = very lightly grazed but with both leaves and top shoot in healthy growing form and 0 = ungrazed seedlings (fenced seedlings), (d) canopy cover, (e) leaf area index and (f) foliar N concentration.

Analysis of Grazing intensities (Figure 12) by grouping oak recruits into heavily grazed, grazed and not grazed, showed strong effect of grazing on the LMA of oak seedlings. LMA values were significantly higher for the heavily grazed seedlings when compared to lightly grazed seedlings or un-grazed fenced seedlings (in both cases $p < 0.001$, one-way ANOVA, Figure 12).

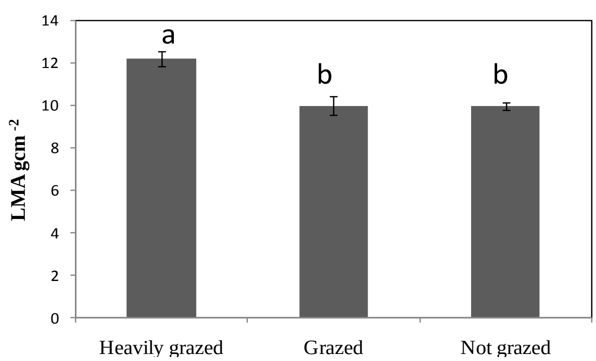

Figure 12. Leaf Mass per Area (LMA, gcm^{-2}) of leaves of seedling grazed with different grazing intensities; Heaviy Grazed= top shoot missing and leaves completely stripped off, Grazed= top shoot present and leaves grazed. Not grazed= protected by shrubs and on which no current signs of grazing were seen. Labels indicate results of one-way ANOVA tests, different letters indicate significant difference. Bar represents standard error of the mean.

4.8. Composition of shrub and herb due to grazing and fencing

The shrub species in the study plots mainly consisted of evergreen species of *Yushania microphylla, Pieris formusa, Daphne bholua* and *Berberis aristata.* There are also few individuals belonging to the deciduous life forms such as *Viburnum foetidum, Rosa sericea*, *Enkainthus deflexus* and *Rubus* sp. The composition of shrubs in grazed and fenced plots was easily differentiable. In the fenced plots where grazing was excluded there were very thick and tall growth of the bamboo *Y. microphylla*, while in the unfenced plots this bamboo species was grazed by cattle and yaks resulting in a highly significant reduction in height as well as the percent coverage (one-way ANOVA, $F_{1,10}$=32.49, $p<0.001$, Figure 13A). In all the fenced plots we recorded very thick growth of *Yushania* reaching as high as 5 m and as many as 30-50 culms in a square meter. *Yushania* covers about 70-90% of the total shrub layer in the fenced plots. In the grazed plots *Yushania* was reduced to about 1 m height and coverage of 10-20 % as a result of grazing. Grazing by animals helped to control the over growth of this bamboo species (Darabant, 2007). Being a fast spreading bamboo the whole ground gets occupied by this bamboo if not controlled. Such growth of this bamboo forming blankets of bamboo leaves on the forest floor will not only have difficulty for seeds to find the mineral soil but also will face severe competition for light and soil nutrients for the growing recruits in the long run. On the other end, unpalatable shrubs which are not grazed by the animals increased greatly in the unfenced plots. Controlling the tall growth of bamboo by grazing animals could have provided the optimum growing conditions of light and space for these light demander shrub species. *D. bholua* and *B. aristata* was significantly larger in the unfenced plots (one-way ANOVA, $F_{1,10}$= 8.27, $p < 0.05$ and $F_{1,10}$= 6.1, $p < 0.05$ respectively, Figure 13A) than the fenced plots. *P. formusa* another unpalatable shrub was also abundant in the unfenced plots however, there is no statistically significant difference in the percent cover between the unfenced and the fenced plots (one-way ANOVA, $F_{1,10}$=0.73, $p > 0.05$, Figure 13A). We also recorded a total of 31 species of herbs from the study area of which the dominant 5 species were shown in Figure 13B. There was however no significant difference in the herb density between the unfenced grazed plots and fenced plots (Figure 13B).

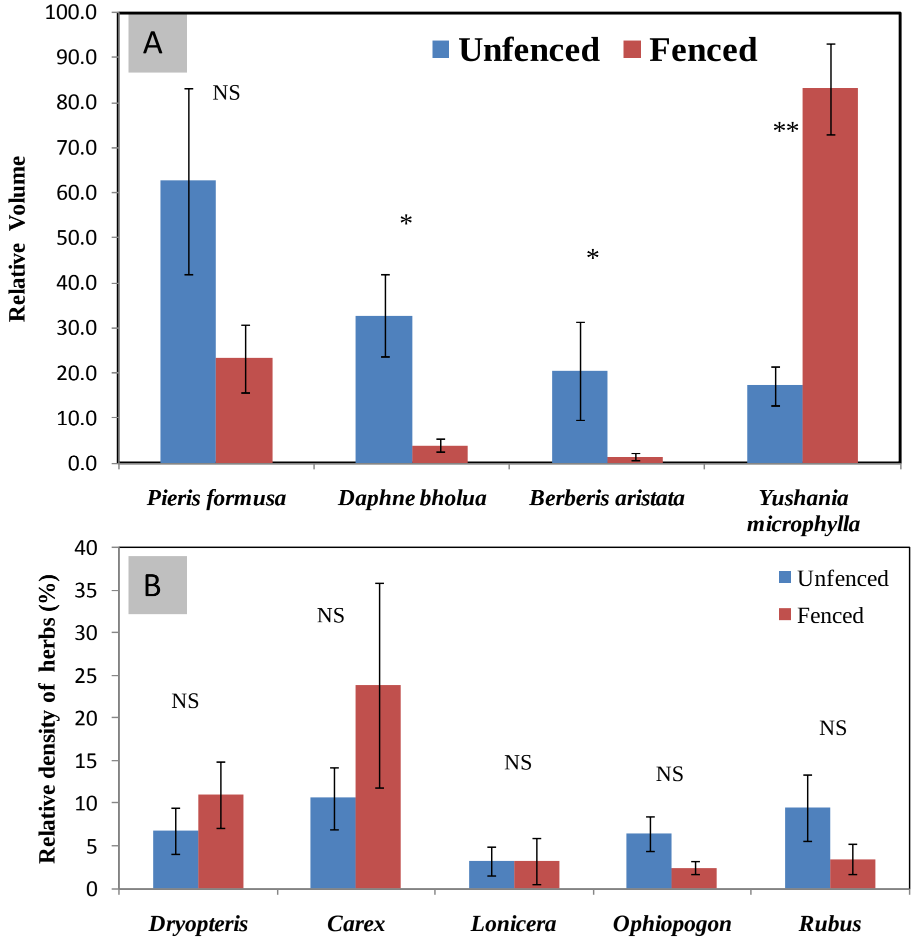

*Figure 13. (A)Relative volume (%) of dominant shrub species in unfenced and fenced plots and (B) relative density (%) of dominant herb species in unfenced and fenced plots. Labels indicate results of one-way ANOVA tests, **P < 0.001, *P < 0.05, NS not significant. Bar represents standard error of the mean.*

4.9. Effect of environmental variables on the regeneration of tree seedlings

The effect of five environmental variables namely soil moisture (SM), soil hardness (SH), mean openness of the canopy (MOPEN), slope and grazing on the regeneration of tree recruits were studied through canonical correspondence analysis (CCA, Figure 14). There was a significant effect of environment variables on the growth, survival and distribution of seedlings and saplings (Monte Carlo test, F-ratio= 2.77, $p < 0.05$) which is indicated by a clear grouping of species along the environmental variables. Recruits of species like *Taxus baccata, Pinus wallichiana and Picea spinulosa* that are unpalatable to grazing and which require sufficient amount of light were grouped together along the axis with increasing mean opening of canopy and grazing intensity (left corner of axis 1). Grazing controlled the growth of competing vegetation especially bamboos. Therefore, the abundance of above listed species were positively correlated with mean openness of the canopy as well as grazing intensity. Species like *Rhododendrons* and *Ilex* occurred on the exposed and dry sites with compacted soils and thus is negatively correlated with soil moisture but positively correlated with soil hardness. *Q. semecarpifolia* recruits preferred slopes and are positively correlated with slope. The recruits grew and survived well under the shades and were susceptible to increasing grazing pressure, so their abundance was negatively correlated with increasing mean openness of canopy as well as with increasing grazing intensity. Deciduous broadleaf species namely *Corylus ferox* and *Acer campbellii* preferred moist locations and hence they are associated soil moisture axis. The length of each arrow of the environmental variable signifies the degree of its effect on the distribution of tree seedlings. All the environmental factors played major role in species distribution with mean opening, slope and soil moisture having the longest arm followed by grazing intensity and lowest by soil hardness arm.

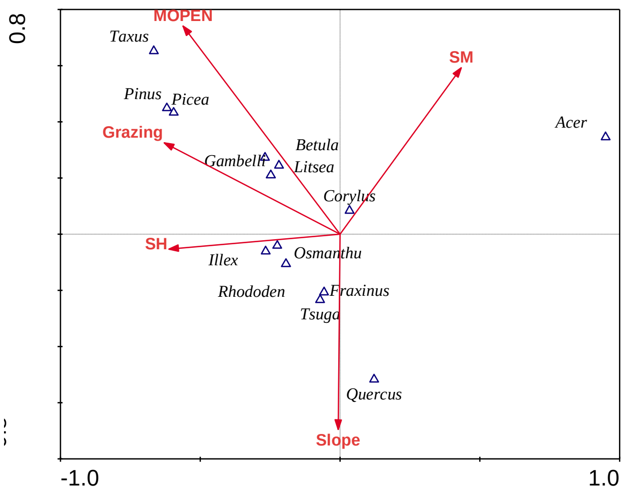

Figure 14. Results of canonical correspondence analysis (CCA). SM refers to soil moisture, MOPEN= mean opening of the canopy and SH= soil hardness.

5. Discussions

5.1. Distribution of tree species and recruits along the local topography

Local topographies can explain up to certain extent the distribution of species and variation in the forest structural features. The Himalayas are blessed with a variety of forest types that has fascinated many researchers to study the distribution of vegetation types along the altitudinal and climatic gradients (Vetaas, 1998, Wangda & Ohsawa, 2006; Choden, 2010). While these studies were carried on a larger scale covering several forest types, the role of small scale local topographies like ridge tops, valleys and slopes on the distribution of species were not well studied despite being one of the important factors affecting the distribution of plant species in the mountain forests (Enoki, 2003). Small scale land features like north or south facing slopes or topographical gradients like convex and concave landscapes have a greater role in distribution of vegetation types and forest structural features (Enoki, 2003; Wangda et al., 2010). The variation in topographic features can cause variations in the soil moisture, light availability, drainage and nutrients availability (Zak et al., 1991; Enoki, 1997). Distribution of species to a particular site largely depends upon the preferable habitat qualities available.

Higher species richness and diversity on the ridge and slope than in the valley reveals the importance of light availability, good drainage and natural disturbances which are more frequent on the ridges and slopes than in the valleys (Enoki, 2003; Tashi, 2004). More numbers of light-demanding species like *Rhododendron arboreum*, *Pinus wallichiana* and *Taxus baccata* are thus recorded on the ridge tops and slopes. Deciduous broadleaf species like *Acer*, *Betula* and *Corylus* spp. preferred the cool and moist sites in the valleys. On the contrary, evergreen species like *Quercus*, *Osmanthus* and *Ilex* spp. were more abundant on the slopes. Enoki (2003, 2004) reported that slopes and concave land features are unstable and natural disturbances like landslides promote the regeneration of evergreen broadleaf species. The results seem to indicate that *Quercus semecarpifolia* seedlings require disturbances or newly exposed soil for successful regeneration. On the other hand, the conifer, *Pinus wallichiana* seedling and sapling densities were recorded higher on the ridge even though there is no mother trees in the vicinity. The old pine forests from the surrounding lower elevation shed abundant seeds of *P. wallichiana* which are very strong colonizers (Mong, 2006) and the changing climatic conditions due to global warming might be favoring the survival and growth of this species higher up the elevation above its natural distribution range. There are no studies done on the upward movement or encroachment of *Pinus wallichiana* to higher elevations (Royal Government of Bhutan, 2011) and requires further study to ascertain the relationship between this unique distributional pattern of *Pinus wallichiana* to changing climatic conditions in the Himalayas.

5.2. Effects of grazing and fencing on the tree recruit density and composition

Distribution of mature trees did not differ between grazed and fenced plots. The fencing was only established in 2000 and the effect of fencing on the mature trees was difficult to determine. Never the less, it is a good indication that the seed rain (Linhart & Whelan, 1980), which is the main source of natural regeneration, was uniformly distributed between the grazed and fenced plots. The abundance of seedling and saplings in fenced and unfenced plots was however not similar with the distribution pattern recorded for the mature trees. The percent similarities between the abundance of mature trees and young recruits were higher in the unfenced plots than the fenced plots. Significant increase of the bamboo *Yushania microphylla* after fencing must have prevented the regeneration of canopy tree species like conifers and rhododendrons by competing for light, nutrient and space (Darabant et al., 2007; Gratzer et al., 1999).

The sapling numbers in the fenced plots in 2011 was two times higher than the total count of saplings in grazed (unfenced) plots. Seedling density of canopy dominant *Q. semecarpifolia* was abundant in both grazed and fenced plots in 2000 which reflects a mast cropping or a good seeding year in the previous year or one year earlier. The germination rate of this oak is very high, 95%, but, unfortunately the viability of the seeds lasts for a very short period (Shrestha, 2003) and the acorns are large and heavy which limits their distribution range and hampers their regeneration ability. Every falling seed is likely to germinate however, finding mineral soil and transforming into young seedlings and sapling phase will depend on finding a right place or “safe sites” (Christian, 2005). This is especially true for the oak seedlings and saplings which are exposed to grazing. Growing on safe micro-sites in association with unpalatable shrubs was found to have a natural facilitative effect on oak recruits (Callayway, 1998, Christian, 2005). Association with thorny and unpalatable shrubs also acted as a bio-fence and protected oak recruits from herbivory (Tashi, 2004). The higher number of oak saplings in the fenced plots also supports the above findings.

There is high mortality of oak seedlings in both unfenced and fenced plots which resulted in underrepresentation of oak saplings indicating that most seedlings did not find a safe micro-site to grow. This is consistent with the observations in other Himalayan oak forests (Singh & Singh, 1987; Vetaas, 2000). Studies in Nepal related the absence of oak regeneration under its own canopy to climate change (Upreti et al., 1984), insufficient canopy disturbances (Metz, 1997) and to fire and indiscriminate annual lopping of trees for fodder that did not give enough seeds (Singh and Singh, 1992; Shrestha and Paudel, 1996; Vetaas, 2000). From our study, we found that the count of saplings increased significantly in fenced plots than the grazed (unfenced) plots. Further, there were neither signs of fire nor indiscriminate lopping of oak trees in the study area which revealed that over-grazing could be the single most important factor that is preventing the recruitment of seedlings into sapling and tree phases.

Oak is highly palatable and a preferred fodder by the grazing animals (Roder, 1992) and in the absence of fence, freely grazing animals limited their count by repeatedly browsing and trampling. Tashi (2004) and personal field observation verified these assumptions as we found healthy saplings growing even in the unfenced plots when they are associated with or protected by the thorny, unpalatable species *Berberis aristata*. But soon after the sapling’s growth surpasses the height of *Berberis* they are heavily grazed and browsed to the extent that the entire top shoot of the sapling lying above the *Berberis* height is grazed (Figure 15).

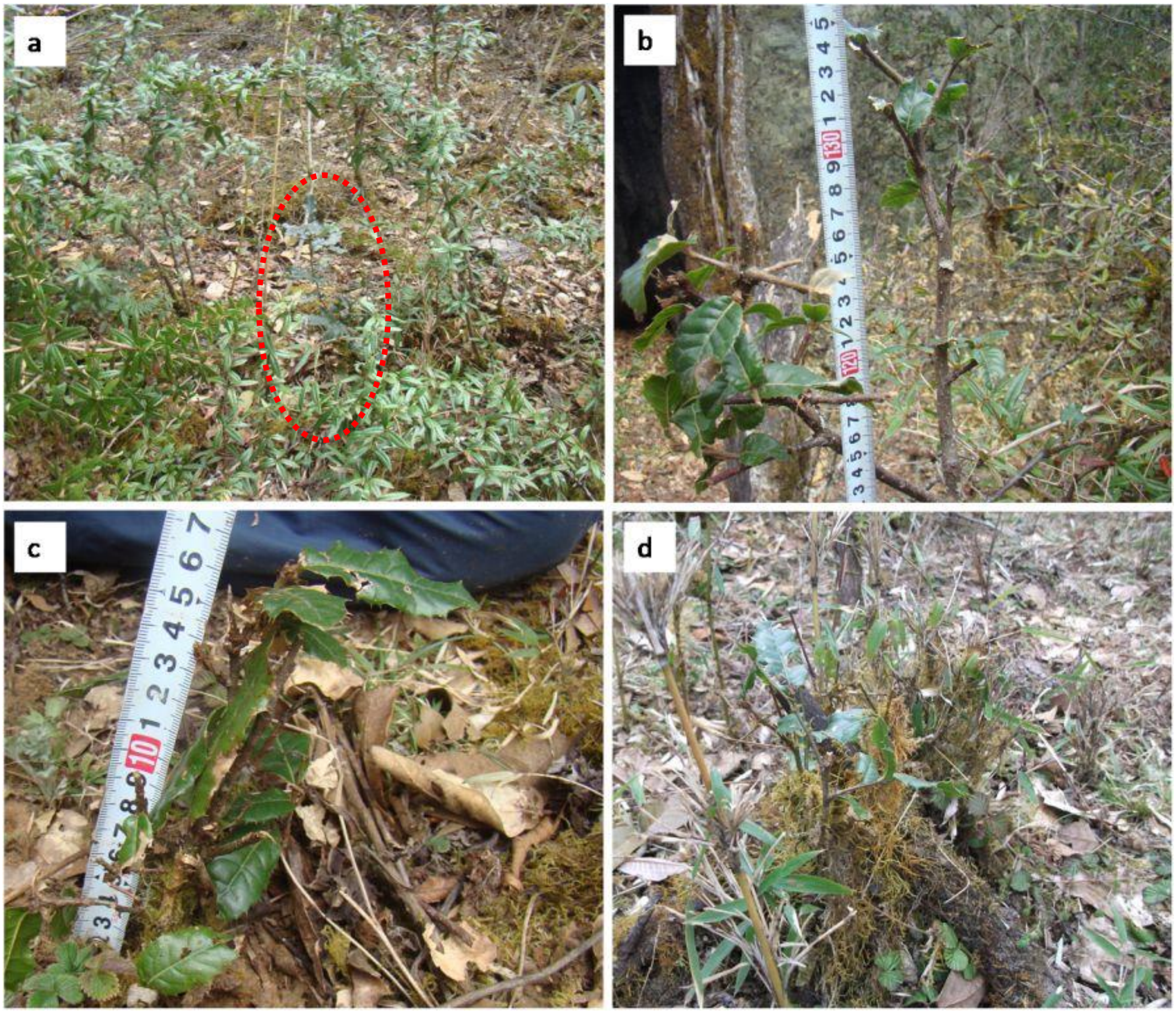

Figure 15. Picture showing the oak saplings growing in unfenced plots. a) A healthily growing oak sapling (in red circle) protected from grazing animals by Berberis aristata (surrounding shrub) b) Heavily browsed sapling with top shoot missing when the seedling grows above the height of the Berberis and c) Heavily browsed saplings when not associated with any protective covers d) Heavily browsed seedling even when they are surrounded by palatable bamboo.

Fencing has a strong positive effect on the abundance of sapling of *Q. semecarpifolia.* The positive effect of fencing could be seen from two perspectives. First and the most obvious reason is that fencing protected the saplings from being grazed and trampled by the animals and allowed them to grow normally. The other could be the effect of tall growth of bamboos in the fenced plots that could have protected the oak seedlings by providing shade and preventing desiccation from direct sunlight. *Quercus semecarpifolia* is a very unique species which survives better in shaded sites in the early stages of development and is negatively related with canopy opening (Vetaas, 2000; Tashi, 2004). Our results also showed that canopy cover is positively related with abundance of *Quercus semecarpifolia* regeneration and therefore supports the above findings. However, as oak seedlings establish and grow further into the older stages of development, enormous amount of sunlight is required (Vetaas, 2000). This is not often provided in the natural forests with small canopy openings and as such there is heavy mortality of oak saplings in these forests leading to a condition where there are many seedlings provided by the matured mother trees but without or with very few saplings (Vetaas, 2000; Shrestha, 2003; Tashi, 2004). In this study too, we observed that there is complete absence of oak individuals in between the diameter classes 1.5 cm and 20 cm reflecting the underrepresentation of older saplings and young trees in this forests (Figure 16). Many authors are of the opinion that the failure of oak regeneration is an attribute of low disturbances that lead to only small canopy openings and that larger openings are required to regenerate this species (Singh, 1997, Shrestha, 2003). In the unfenced forest with fairly open canopy, recruitment of oak into sapling stages were higher because grazing by animals controlled the height of bamboos and provided optimum light requirements for the oak seedlings. However, along with the bamboos, oak seedlings and saplings are also grazed repeatedly which doesn't give opportunity for this species to grow and survive. This is especially true when the grazing pressure is high (Figure 15). The failure of regeneration of the oak, *Quercus semecarpifolia* seems to be very complex involving the interaction of natural as well as anthropogenic causes and is no wonder an "environmental semi-surprise" (Singh et al.,1997, p. 371).

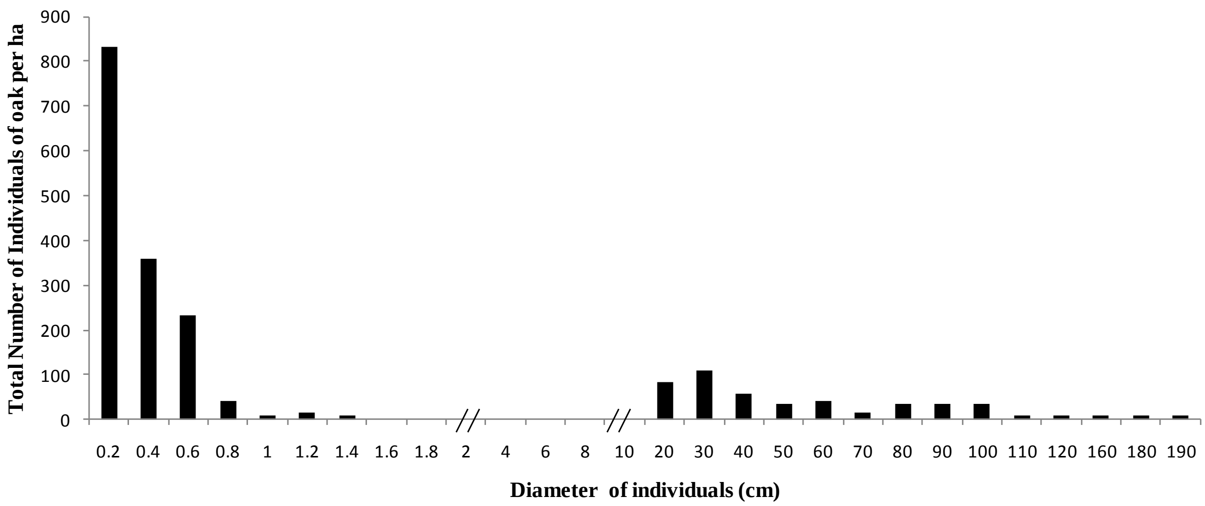

Figure 16. Current status of brown oak recruitment in the study area according to the diameter distribution classes. Figure shows the absence of individuals between 1.5cm to 20cm dbh. Diameter of individuals with height less than 1.3m was measured at collar region and diameter of individuals above 1.3m height was measured at breast height. Data pooled from unfenced and fenced plots.

5.3. Effect of grazing and fencing on the seedling morphological traits

Seedling and sapling height in the fenced plots differed significantly from the unfenced grazed plots. Mean height of the fenced plot recruits were two times larger than the mean height of the unfenced plots. Seedling survival into sapling stage was greatly enhanced by fencing which is indicated by presence of more number of individuals in the upper height classes (Figure 9a). The collar diameter distribution of recruits did not differ between the two treatments indicating the least impact of grazing on the seedling diameter. The recruitment of seedlings was higher in the lower height and collar-diameter classes but the recruitments further into the upper classes decreased greatly in the grazed unfenced plots (Figure 9) except for few species (which are not grazed) where the recruitment into upper classes were higher in the unfenced grazed plots (Figure 9 d). Grazed seedlings like oak and hemlock showed higher recruitment in the young height classes but their numbers decreased significantly higher up the classes (Figure 9b and 9c). They are therefore grazing decreasers. On the other hand recruitment of unpalatable species such as rhododendron to upper height classes was continuous and the counts in the upper height classes were even higher in the grazed unfenced plots (Figure 9d), they are therefore grazing increasers. The results show that height growth and shoot elongation of seedlings and saplings were affected by herbivory (Adams, 2000). Pearson correlation between height and collar-diameter of the recruits between unfenced and fenced plots supported this assumption. There was a significant correlation between the recruits' height with collar-diameter in both unfenced and fenced plots however, the strength of correlation was significantly higher in the fenced plots than the grazed unfenced plots (Figure 9h) which reflects that repeated grazing limited the height growth of recruits in the unfenced plots while collar diameter remained unchanged in both the treatments. This resulted in a weaker correlation between height and collar diameter in the unfenced plots than the fenced plots where both height growth and collar diameter increment was not disturbed by grazing.

Some authors are of the opinion that grazing has beneficial effects to the plants by encouraging "vigorous regrowth", increased total biomass and seed production leading to "herbivore optimization" and "plant animal mutualism" (Owen & Wiegert, 1976; Paige and Whitham, 1987). While many others (Ellison,1960; McNaughton,1986 & Belsky,1987) argue and relate the findings misleading or "red herring" and are due to crude experimental design, inadequate method, or cover short time period which reflects only the initial stimulation in herbage growth (Belsky, 2003). In both the cases however, the nature of plant species and their resistance to herbivory differs widely amongst the species which needs to be considered in order to define the harmful or beneficial effects of grazing. From our study, height growth of the oak seedlings and saplings were significantly reduced by grazing. In the grazed plots more than 60 % of the oak recruits were heavily grazed with top shoot completely missing. Over grazing of seedlings significantly reduced the recruitment of seedlings in to sapling stage in the unfenced plots. Most of the grazed recruits did not recover as indicated by low count of individuals in the unfenced grazed plots. There was no sign of increased growth rates or "grazing optimization" of oak seedlings and hence did not indicate the beneficial effects of grazing on oak recruits. Our study is in agreement with authors like Ellison (1986) and Belsky (2003).

None the less, a similar study in a conifer forest of Bhutan by Darabant et al. (2000) reported the significant increase in the recruitment of individuals in the upper diameter classes in the unfenced grazed plots which is contradictory with our findings. The main reason for this difference may be due to variations in the forest types, the kind of species present and extent of their grazing resistance. As their study were carried in the conifer belt with tree species that are more light demanding and comparatively grazing tolerant, grazing could have helped to reduce shrub growth and promoted conifer regeneration. Our study was concentrated in the temperate broadleaf forest where the canopy dominant species, *Quercus* spp. are susceptible to grazing.

Apart from morphological characters like height and collar diameter, grazing also had important implications on the leaf surface area, leaf mass per area (LMA) and foliar N content as indicated by this study. The amount and the extent up to which the plants can withstand and recover from damaged caused by herbivory will determine the degree of their survival. Seedling leaves of grazed unfenced plots showed presence of more surface spines, high thickness and significantly reduced leaf area than the leaves of fenced plots. We assumed that these changes are as a result of morphological reinforcement to protect from environmental stresses particularly grazing. The presence of surface spines in the leaves is a clear sign of defense from herbivory (Wright & Cannon, 2001) and thick leaves is an indication of high accumulation of secondary compounds that act as a chemical protection from the grazing animals (Chabot and Hicks, 1982). Significant reduction in leaf area due to grazing would have profound implications on plant growth and survival. Lower leaf area would mean reduced photosynthesis and reduced accumulation of carbon reserves. As photosynthetic capacities of plants are strongly correlated with N content of leaves (Mooney & Gulmon, 1979), there is no reason to think why N content were significantly lower in small leaves that are grazed. Casotti & Bradley (1991) reported that herbivory by insects lead to lower level of leaf nitrogen in *Eucalyptus* sp. To defend from grazing and browsing effects the leaves might have diverted carbon from other functions to accumulate herbivory-resistant chemicals that is "metabolically expensive" and increases the thickness and weight of the leaves (Chabot and Hicks, 1982). The outcome is a thick and heavy leaves with low surface area. This gives a significantly high leaf mass per area (LMA) of grazed seedlings compared to the not grazed seedling leaves as indicated by our study (Figure 12). Wright et al. (2002) reported that higher values of LMA of plants are associated with dry and nutrient poor habitats which allow species in stressful conditions to survive better and achieve longer leaf life span while authors like Ogaya & Penuelas (2007) related higher LMA with environmental stress factors like rainfall, temperature and solar radiation. Complete absence of saplings and young trees (Figure 16) as well as wide variations between LMA of grazed and un-grazed recruits growing under the same climatic conditions from our study indicated that higher values of LMA need not necessarily help the seedlings to survive stressful conditions nor are attributed to climatic stresses as assumed.

Higher values of LMA was positively correlated with grazing intensities with larger values of LMA for heavily grazed recruits and lower values for lightly and non-grazed recruits (Figure 12). There could be two possible outcomes: 1) heavily grazed seedlings with high LMA may both lose physiological functions and die or 2) Poor nutrient content and rich secondary compounds in the leaves render the oak recruits less palatable to grazing animals. Since, the resistance shown by the oak recruits in our study plots were not strong enough to defend from the large herds of grazing animals, we assume that the former is possibly occurring where the oak seedlings under grazing stress were dying and recruitment into sapling phase is not currently occurring in the grazed plots. Reduction in the grazing pressure could possibly lead to escape of some individuals from being repeatedly browsed or grazed.

5.4. Effect of grazing and fencing on the shrub layer cover and composition

The undesirable result of exclusion of grazing animal due to fencing is the over-growth of bamboos (Darabant et al., 2007) and brambles (Linhart and Whelan, 1980). Our study also indicated similar results. There were very thick and tall growths of the bamboo, *Yushania microphylla* in the fenced plots. The growth of bamboo in unfenced plots is reduced by almost five times due to grazing and trampling by animals (Figure 13A). This thick growth of bamboos in the fenced plots appeared to reduce the regeneration of tree seedlings especially those of conifers through light, water and nutrient competition (Darabant et al., 2007). Our study also showed similar findings. Relationship between regeneration of conifer and oak seedlings and relative volume of bamboo is shown in Figure 17. Conifers are generally light demanding species and have a negative relationship with increasing bamboo cover. To promote the successful regeneration of conifers removal of bamboos manually or through controlled grazing in these forests might be necessary (Darabant et al., 2007). On the other hand, regeneration of the oak, *Quercus semecarpifolia* seemed to be more favorable under the thick growth of bamboos. There were as many as three times more oak recruits in fenced plots with thick bamboo cover compared to unfenced grazed plots with less bamboo cover. Oak being a shade-loving species in the early years of growth prefers and grows well under shade which might be consistent with the findings reported by Tashi (2004) and Veetas (2000). The positive shading effects resulted in a weak positive relationship between number of oak recruits and bamboo growth. However, the relationship is not very clear because in the grazed plots both oak and bamboos are palatable and browsed simultaneously. But there are reports that oak recruits require enormous amount of light in the latter stages of growth and devleopment. This requires considerable amount of canopy opening which is currently not provided by the small disturbances (Singh, 1997). As such there are not enough saplings and small trees of oak in these forests (Singh, 1997; Veetas, 2000; Tashi, 2004) which is a great concern. Competition from thick and tall bamboos might also hamper the regeneration and recruitment of oaks and other tree species (Nomiya et al., 2002, Darabant et al., 2007). Our fenced plots were established only in 2000 and brown oak being a very slow growing species, there were no matured saplings or young trees with height greater than 1.6 m. In the unfenced plots all the saplings were grazed heavily which prevented the successful recruitment of oak. Therefore at this stage, it is too early to judge the ill-effects of bamboo on the recruitment of brown oak saplings to young tree and upper phases. None the less it provides a good opportunity to understand the role of bamboo on regeneration and recruitment of oak by continuing the current experimental research and involving treatments that allows the removal of bamboos manually from the fenced plots.

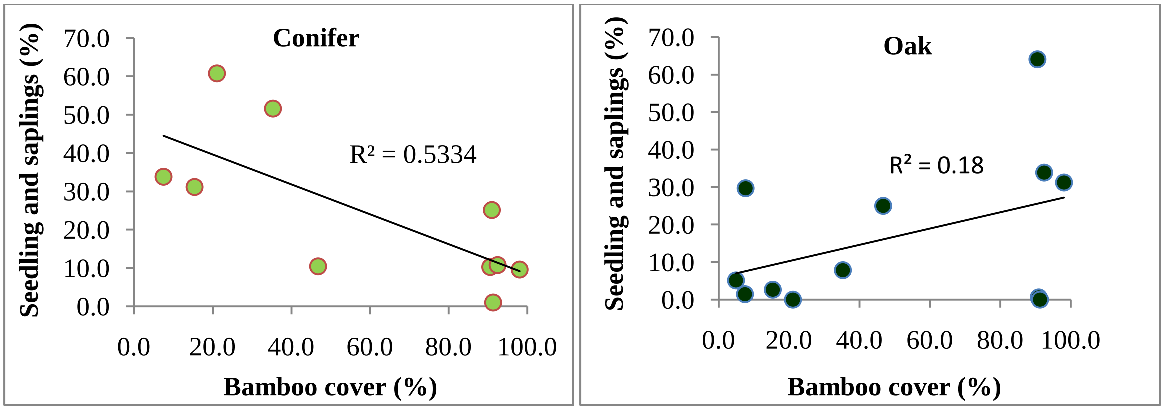

Figure 17. Effect of bamboo cover (relative cover) on the regeneration of conifers and oak.

Certain shrubs like *Berberis aristata* and *Daphne bholua* behaved as grazing increasers. Their numbers significantly increased in the grazed plots compared to fenced plots. The main reason for the increase might be because they are grazing resistant or non-palatable. Grazing of bamboos by animals increased the light and space for them to grow. Increased unpalatable shrubs in the grazed plots have some advantages as they acted as nurse crop and as a bio-fence by protecting the oak recruits from desiccation effects and grazing animals respectively (Figure 13A and Figure 15).

6. Conclusion

The regeneration of the temperate broadleaf forests particularly brown oak is a very complex process and is attributed to many factors. Being a late successional species it does not possess any advantages of fast colonizers. The acorns are recalcitrant in nature and are short-lived; huge and heavy which limits the dispersal range. The unique regeneration requirements of the species: considerable shade in early stages of seedling establishment and strong overhead sunlight in later growing phase results in considerable mortality of young seedlings as these requirements were mostly not available in the natural forests. Overgrazing by domesticated animals which freely graze in these forests browse the established seedlings and saplings preventing further recruitment into upper growth phases. The sapling phase is the most susceptible stage for the large number of browsing animals as they become more prominent and visible to the grazing animals.

The factors like overgrazing that completely wipes the saplings and prevents the recruitment of oak into the young trees and upper canopy layers are more severe than the natural factors which cause heavy mortality of seedlings. Assuming that only one seedling is enough to replace one mother tree (Lorimer et al., 1994; Vetaas, 2000; Tashi, 2003); the effect of mortality of seedlings is far more acceptable than the failure of recruitment into respective upper growth phases due to over-grazing.

There are indications that exclusion of grazing by fencing has a positive effect on the regeneration and growth of oak recruits particularly the saplings. However, very thick growth of bamboos after fencing presents a huge risk for seedling establishment and recruitment in future. The study findings reveal that successful regeneration of oak can be expected in places where the overhead sunlight is optimum and where grazing pressure is low. To ensure these factors are met in the broadleaved forests dominated by brown oak, manipulation of canopy opening and grazing levels needs to be considered while managing these forests. Manual removal of thick growth of bamboos in the fenced plots could be an option in future to study the regeneration performance of oak recruits as well as to understand the role of bamboos in oak growth and survival.

We suggest that controlled grazing by reducing cattle population through improvement of livestock breeds (Norbu, 2002; Buffum, et al 2008) could be a viable option in promoting adequate regeneration of oak as well as sustainably managing the temperate broadleaf forests of Bhutan Himalayas. While the continuation of the current study is necessary, the study also stresses the need to further expand such experimental study to other broadleaf forest types of Bhutan. Many of the broadleaf forests of Bhutan are least studied and there are no adequate information on grazing and forest regeneration. Replication of similar studies by deploying fenced and adjacent comparable unfenced plots will give a better idea of grazing and regeneration interactions on a wider scale.

There are also evidences of failure of oak regeneration in the English woodlands due to defoliation by caterpillars. There are no studies done on the impacts of browsing or defoliation by invertebrates on *Quercus* spp. in the Himalayas and therefore would be interesting to study and compare grazing and browsing effects of both vertebrate and invertebrate animals on the regeneration of brown oak.

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Regeneration of dominant tree species in a temperate broadleaf forest of Bhutan Himalayas with special reference to grazing and fencing

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I. Introduction

Temperate broadleaf forests forms one of the important forest types of the Bhutan Himalayas and are very strongly associated with the subsistence hill agriculture and livestock rearing. Forest grazing by domestic cattle is an age-old tradition and is intermingled with the sustenance and livelihood of people in Bhutan (Norbu, 2002). However, in the last few decades or so, the dependence on these forests occurred on a large scale due to rapid growth in livestock population resulting in overgrazing of the broadleaf forests. The entire forest in Bhutan is commonly grazed by domestic, migratory herds of cattle and wild animals. Forest provides the richest source of fodder, and broadleaf forests are widely preferred for grazing due to rich fodder resources.

Broadleaf forests in the Himalayas harbor the remainder of a climax flora and fauna and are important from the conservation point of view. Studying and understanding the effects of grazing on regeneration are very important to manage these forests sustainably. Many researchers from all over the world have reported the poor and uncertain oak regeneration in these forests. Broadleaf forest in Bhutan is not an exception and many researchers have often related the problematic regeneration to cattle grazing (Norbu, 2002; Ijssel, 1990; Wangda and Ohsawa, 2005). However, there is no quantitative data to show the relationship between the grazing and regeneration problems of oaks. This study is therefore carried out to assess the impact of grazing on the regeneration of temperate broadleaf forests with special focus on brown oak (*Quercus semecarpifolia* Sm.) and grazing activities which are likely to change their character. The study is expected to provide a knowledge-base for scientific understanding of broadleaf forests in Bhutan.

II. Materials and Methods

The study was conducted in a temperate broadleaf forest at Chimithanka, under Thimphu district of Bhutan, at an altitude of 2800 to 3100m asl. This area belongs to the vegetation zone of evergreen oak forests (Sargent et al., 1985). Six square plots of 10 m × 10 m were fenced to prevent animal grazing, and another 6 comparable 10 m × 10 m plots were set in adjacent forest outside the fence (unfenced). The fence was established in 2000 and vegetation survey was done in summer of 2011. Regeneration survey and abundances of mother trees that produce seeds for natural regeneration were determined through tree layer survey in 15 m radius circular plots from the centre of each 10 m × 10 m plot.

III. Results and Discussions

The broadleaf forest was very diverse with 35 tree species, 8 shrub species and 32 herb species recorded in total. Regeneration of brown oak (*Q. semecarpifolia*), the canopy dominant species, was poorly represented at the sapling and small tree stage. As reported in other Himalayan oak forests, heavy mortality of seedlings and saplings might be responsible for lower recruitment of oaks into upper classes

(Thadani & Ashton, 1995; Vetaas, 2000). Overgrazing by livestock in the study area (Department of Forestry, 1992) appeared to hamper recruitment of brown oak. Due to taller height and easier visibility, saplings were more browsed and damaged than the seedlings by grazing animals (Nomiya et al., 2002, Figure 1). As a result the number of saplings in the unfenced plots was significantly reduced compared to fenced plots (Figure 1). Grazing had a very significant negative effect on the height growth of the oak recruits (Figure 2); however, the collar diameter was not affected by grazing (Figure 3). The diameter-height relationship of oak recruits therefore showed that repeated grazing resulted in depression of height growth in unfenced plots (Figure 4). From our results, fencing has shown to improve the regeneration, establishment and growth of brown oak; however, fencing and complete exclusion of animals also resulted in very thick and tall growth of the bamboo *Yushania microphylla* (Figure 5). This might cause another problem to regeneration of oak through competition for light, water, space and nutrients in future (Gratzer et al., 1999; Nomiya et al., 2002, Darabant et al., 2008). It is recommended to continue the experimental research with treatments involving removal of bamboos from the fenced plots to get a better understanding of brown oak regeneration. For successful regeneration and recruitment of brown oak, the population of grazing animals should be reduced in the broadleaf forests of Bhutan Himalayas.

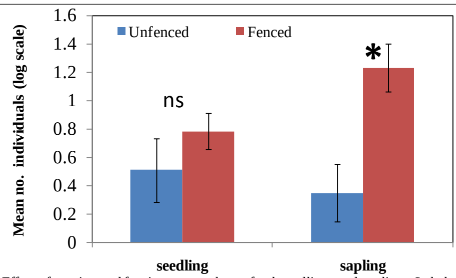

*Figure 1 Effect of grazing and fencing on numbers of oak seedlings and saplings. Labels indicate results of one-way ANOVA tests, *P < 0.05, ns= not significant. Bar represents standard error of the mean.*

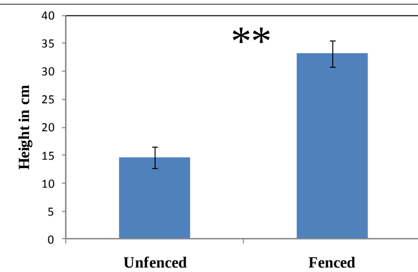

*Figure 2 Effect of grazing and fencing on height growth of oak recruits. Labels indicate results of one-way ANOVA tests, **P < 0.001. Bar represents standard error of the mean.*

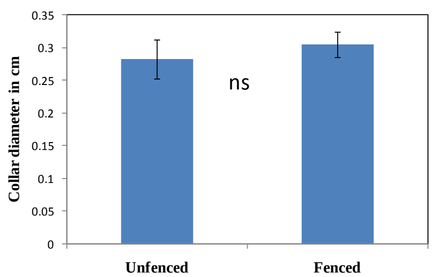

Figure 3 Effect of grazing and fencing on collar diameter of oak seedlings and saplings. Labels indicate results of one-way ANOVA tests, ns= not significant. Bar represents standard error of the mean.

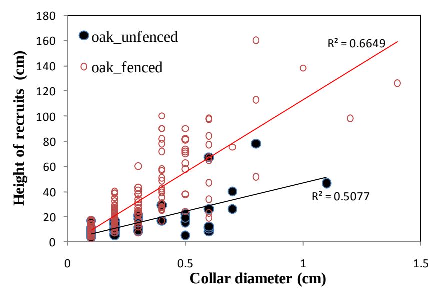

Figure 4 Diameter- height relationship showed a depression in the height growth of grazed oak recruits.

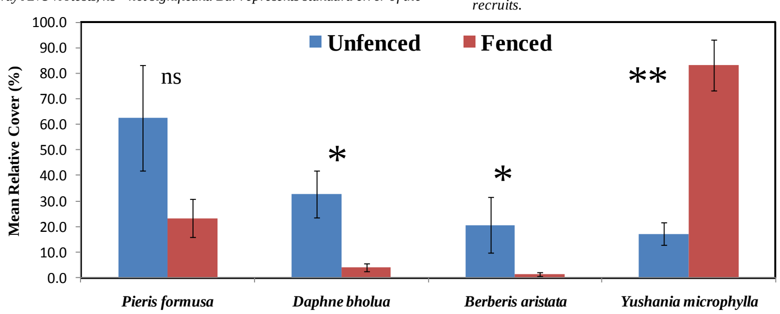

*Figure 5 Effect of grazing on shrub composition . Labels indicate results of one-way ANOVA tests, **P < 0.001, *P < 0.05, ns = not significant, Bar represents standard error of the mean.*