Regeneration Status and Soil Nutrient Content in Burned Blue Pine Forest in Thimphu, Western Bhutan

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ARTICLE INFO
Keywords: Blue Pine; Diversity; Dominance; Forest Fire; Regeneration.

ABSTRACT
A forest fire and human disturbances are a major threat to regeneration and forest health. This study was conducted in western Bhutan in Thimphu, above Depsi. The Objective of the research was to assess diversity, species richness, regeneration, and soil nutrients content (N, P & K) along the altitudinal gradient after a forest fire. A total of 30 standard plots of size 20x20 m plots were established in the burned blue pine forest systematically, along the altitudinal gradient. Systematic random sampling was adopted. Sampling was carried out at three different altitudinal zones that is; lower (2500-2700 masl), middle (2701-2900 masl), and higher (2901-3100 masl) altitude. A total of 1703 individual plants belonging to 21 families were recorded from the study. The diversity, regeneration, and soil nutrient increased till mid-altitude and decreased with further increase in altitude, making a unimodal, hump shape pattern. The regeneration, soil nutrient, and diversity were higher in the gentle slope and the slope facing southeast due to the longer duration of sunlight exposition. The regeneration and diversity of vegetation showed a positive correlation with aspect, carbon, phosphorous, and nitrogen (p<.05). The diversity and regeneration after a fire was affected by environmental factors, especially soil nutrients, aspect, and slope. The study forms the baseline data for the forest manager to sustainably restore the forest after a fire. Species resistant to fire can be chosen for forest restoration. It can also be incorporated in management plans for sustainable management and conservation of mountain blue pine forests.

INTRODUCTION
Bhutan has 70.46% forest cover out of which 51.44% forest is under protected area (National Park, Wildlife Sanctuary, and Strict Nature Reserve & Botanical Garden) in the country (Pem, 2016). The forest type is classified based on altitude, precipitation, and dominant vegetation (National Forest Inventory (NFI, 2016). The Blue pine forest in Bhutan is found between altitude ranges of 2100-3100 masl with precipitation ranging from 1000-1559 mm annually (National Statistical Bureau (NSB, 2016). The Blue pine forest type is found along with Haa valley, Paro, Thimphu, Bumthang, and Mongar districts of Bhutan.

Forest fires have occurred due to the burning of debris, the agricultural field, and waste pit by local people which had spread to nearby pine forests (Certini et al., 2011). Blue pine forests not only uplift the economic well-being of local people but also protect the steep slopes from landslide and erosion. Blue pine forest provides timber the best quality timber for construction and for making valuable items that provide income to the people. Blue pine forests also provide (Non-Wood Forest Product) NWFP, fodder for cattle, and firewood to the nearby residents (Gempa, 2014). The study carried out in Trongsa, Bhutan (blue pine forest) by Gempa (2014), found that 62% of older trees and
95% of seedlings of blue pine are burned due to soft and thin barks (Gempa, 2014). Wangdi, (2002) reported that in the blue pine forest, Bhutan, forest fire and cattle grazing are considered as the major threats to forest regeneration.

Fire is the main threat to the mortality of trees in the blue pine forest. Blue pine forests are sensitive to a frequent forest fire as it has thin bark (Konsam et al., 2017). The fire occurs in the winter season when the forest floor is dry with very low precipitation (Kuensel, 2016). In the year 2017-2018, the fire destroyed 16,000 acres of forest reserves from 37 forest fires in 12 Districts across the country (Nima, 2018). Atri et al., (2017) reported that species richness, regeneration, and diversity in burned forest areas is low as compared to undisturbed forest. The fire burns all the undercover vegetation and seedlings decreasing the soil quality, which exerts pressure on the sustainability of the forest (Gupta, 2009). Soil erosion, landslides, and floods after the forest fire not only affect the livelihood of people up-streams but also significantly affect the lives of people living down-streams due to an increase in sediments in water bodies (Wangchuk, 2017). For example, the study conducted by Sankey et al. (2017) in the western USA in a fire-prone forest area found out that, the burned areas increase soil erosion rates within watersheds, which can increase sedimentation in downstream rivers and reservoirs. The increase in sedimentation could negatively impact water supply and quality for some communities, in addition to affecting stream channel stability and aquatic ecosystems (Sankey et al., 2017).

The study area was conducted at Depsi, Thimphu with the objective to assess the diversity, regeneration, and soil nutrients content (Nitrogen (N) Potassium (K), and Phosphorous (P)) along the altitudinal gradient after a fire. The study also aims to analyze the relationship between regeneration and environmental factors. The study forms the baseline data for the forest manager to sustainably restore the forest after a fire.

**Materials and Methods**

**Study area**

The study was carried out above Depsi in Thimphu in the burned blue pine forest. The area is located at 27.43392°N, 089.631110°E and 2743878°N, 89.62097° E. The forest is dominated by blue pine (*Pinus wallichiana* AB Jacks) along with some hardy shrubs, perennial and annual herbs. The area was burned twice during the dry winter season in the year 2015 and 2017 and it had lost most of the trees (Pem, 2016). The study area is about 90 acres starting from the low altitude (2500 masl) to the top of the mountain (3100 masl). Anthropogenic activities like grazing by animals, logging of timber, and human settlement at the bottom of the study area can be found.

**Study Design and Sampling**

Systematic random sampling was used to avoid bias in the study (Atri et al., 2017). Sampling plots were laid out systematically with an interval of 100 meters between each plot (Schreuder et al., 1993). Burned Blue pine forest of Thimphu was firstly extracted from the google earth engine and moved into ArcGIS for sampling. The fishnet of size 100 x 100 m was then produced. A total of 30 plots from fishnet was then systematically selected. The coordinates of this plot were then punched into Global Positioning System (GPS) while traveling to the field for plot verification. Plots were randomly selected so that the selected sample plots represent the population and avoid bias (Acharya, 2013). The plot size of 20 x 20 m was established for enumeration of the tree and 5 x 5 m for shrubs (Atri et al., 2017). The regeneration status was enumerated in a plot size of 2 x 2 m laid out within the 5 x 5 m plot (Mughal, 2015).

All trees having a DBH (Diameter at breast height) greater than 10 m irrespective of their conditions was considered as the tree in the plots (NFI, 2016). The trees having DBH of 5 -10 cm were measured as a sapling. The plants with DBH less than 5 m was considered as seedling, herbs, and shrubs (NFI, 2016). The individual seedling of all species was counted from the regeneration plots along with height and mean diameter (Bhat et al., 2015). The study area was divided into three different altitudinal zones; lower (2500-2700 masl), middle (2701-2900 masl), and higher (2901-3100 masl) (Sahu et al., 2019). A total of 30 sampling plots were laid in a systematic manner that is 10 plots in each altitudinal zones.

**Collection of soil sample**

The soil sample was collected from individual plots along altitudinal gradient using augur (radius = 3.5 cm, depth = 30 cm). The soil samples were
collected in a zig-zag way within 0-20 cm of depth (Dorji, 2017). At least minimum of four soil sample in a single plot is mixed together and a composite is taken as the main sample to represent the plot (Sahu et al., 2019). A total of 30 soil samples were collected from individual plots along the altitude gradient in the study area. Around four sub-samples were collected from every plot and made one composite sample for nutrient analysis (Sahu et al., 2019).

**Determination of Soil Nutrient**

Before nutrient analysis, the soil was dried and sieved using a 2mm sieve following Sahu et al., (2019). The total nitrogen was determined using kjeldahl method (Bremner, 1960) and phosphorous through Olsen’s method (Watanabe & Olsen, 1965), potassium by flame photometer, and carbon by Gravimetric method (Moktan and Tenzin, 2016).

**Vegetation analysis**

The species diversity was calculated using the Shannon-Wiener diversity index (Shannon, 1948).

\[ H' = -\sum (P_i \times \ln(P_i)) \]

Where P is the proportion (n/N) of each species, n is the number of individual species, N is the total number of species, Ln is the natural log, and \( \sum \) is the sum of the calculations. Frequency, Density, and Abundance (Maia, 2014).

Relative Frequency = Number of occurrence of species A (frequency)/Number of occurrences of all species A (sum of frequency) x 100

Relative dominance = (Total basal area of species/Total basal area of all the species) x 100.

Density = Total no. of individuals of a species in all quadrants/Total no. of the quadrat in which species occurred.

Abundance = Total number of individuals of a species in all quadrats/Total number of the quadrat in which the species occurred (Maia, 2014).

<table>
<thead>
<tr>
<th>Sl.no</th>
<th>Abundance class</th>
<th>Species abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Rare</td>
<td>1-4</td>
</tr>
<tr>
<td>2</td>
<td>occasional</td>
<td>5-14</td>
</tr>
<tr>
<td>3</td>
<td>Frequent</td>
<td>15-29</td>
</tr>
<tr>
<td>4</td>
<td>common</td>
<td>30-80</td>
</tr>
<tr>
<td>5</td>
<td>Abundant</td>
<td>90+</td>
</tr>
</tbody>
</table>

Sorensen’s similarity and dissimilarity index (Ashtamoorthy, 2014).

\[ \text{Sorensen's similarity} = 2C / A + B \]

Where:

QS = Quotient of similarity (ranges from 0-1)
A and B are the no. of species in sites A and B
C is the no. of species common to sites A and B (Ashtamoorthy, 2014)

Index of dissimilarity = 1 – QS

**Regeneration**

The regeneration status of a species was also determined based on the population size of seedlings and saplings. The plant community with good regeneration will have more seedlings than the sapling (seedlings > saplings > adults).

**Statistical Analysis**

Software like Microsoft word, Microsoft excel, and SPSS version 23 (Pearson’s, Spearman’s rho correlation and linear regression test) was used to analyze data and to see the relationship between regeneration, tree diversity, and soil nutrients of burned area (Mukhia et al., 2015).

**RESULTS AND DISCUSSION**

**Species Composition**

A total of 1703 individual species of 21 families were recorded from the study area. The total individual of trees found was 325, 672 individuals of shrubs and 706 individuals of herbs. A total of 215 trees were completely burned in the study area, which accounts for 155 155 m²/ha. The dominant species found were Pinus wallichiana A B Jacks and Quercus semicarpifolia Sm. With (Important Value Index) IVI of 73.870 and 28.250, respectively. Coriaria nepalensis Wall. was the least dominant tree species with IVI of4.610.
Figure 1. An individual number of trees, shrubs, and herbs in the study area.

The species like Desmodium sp. and Artemisia sp. were dominant species found along with the three altitude range with IVI of 19.330 and 20.130 respectively. The Jasminum sp. was found to be least dominant with IVI of 0.540. The ground cover at lower altitude was dominated by Anaphalis sp. with IVI of 13.520 and in the higher altitude by Pteridium aquilinum L. with IVI of 12.400. Equal distribution of herbs and annual grasses were found in mid-altitude where it was least dominated by Rumex nepalensis Spreng with IVI of 1.020 which was rich at higher attitude.

**Growth Status of Vegetation after Forest Fire**

All the species responded either through germination from seeds or re-sprouting from the vegetative parts present before the forest fire. Trees like *Pinus wallichiana*, *Coriaria nepalensis* and *Populas ciliata* regenerate from seeds and they have very poor regeneration. The tree species like *Lyonia ovalifolia*, *Quercus semecarpifolia* and *Rhododendron arboeum* re-sprout from the burned trees' stem and they have grown faster after a forest fire. *Quercus semecarpifolia* has better growth rate after forest fire with more regeneration from its vegetative parts. This is maybe because of the thick barks which protect the plants from heat or their ability to propagate faster from the stem.

Shrub species like Desmodium sp. and Artemisia sp. regenerate from its vegetative parts and have more regeneration compared to *Rubus biflorus* Buch.-Ham. ex Sm, *Indigofera dosua*D. Don, *Berberis asiaticaDc.* and *Rosa sericea*. The herbs like *Heteropogon controtus* (L) P. Beauv. Ex Roem & Schult, *Phalaris aquatica* (L.) and *Pteridium aquilinum* (L.) Kuhn, *Cymbopogon sp.* have grown after the fire with good regeneration from seeds and some from vegetative parts. The herbs like *Anaphalis sp.*, *Primula denticulate* Sm, *Rubia cordifolia* L. and *Rubia cordifolia*L. were poorly regenerated after the forest fire.

Shrub species had a better response to forest fire compared to herbs and tree species. The vegetation regenerated from seeds (44%) and sprouted mostly (56%). The result concurs with the findings of Graciaab et al. (2002) who reported that re-sprouting tree species grow better and faster as compared to those which germinated from seeds. Frondorf (2018), reported that species re-sprouting from the roots and stems cover the area faster than the pine and climbers species.

**Species Abundance**

The abundance of species indicates the health of the ecosystem. The species like *Phalaris aquatic* L, *Heteropogon controtus*, and *Desmodium sp.* which falls under the frequent class of abundance had a higher abundance of 19.800, 16.167, and 15.714 respectively in the area. The species like *Pinus wallichiana*, *Rubia cordifolia*, *Indigofera dosua* and, *Berberis asiatica* (Abundance value of 2.267, 2.000, 1.500, and 1.500 respectively) have low abundance in the area (Figure 3). These species falls under the rare abundance class indicating poor distribution in the area. The abundance of tree species and shrubs lowered in lower altitudes due to logging activities and anthropogenic activities. Ogwu & Osawaru (2016) reported that species abundance decreases with anthropogenic activities, which indicates that the area needs constant monitoring and surveillance.

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**Figure 1. An individual number of trees, shrubs, and herbs in the study area.**

![Pie chart showing tree, shrub, and herb distribution.]

- **Tree** - **Shrub** - **Herb**
- 326 Tree
- 706 Shrub
- 672 Herb
Figure 2. Similarity and dissimilarity of species along an altitude gradient

The Sorenson’s similarity index showed a higher similar species composition between the lower altitude (2500-2700 masl) and the middle altitude (2701-2900 masl) with 80.9% similar species composition. The study showed a high percentage of species similarity (73.5%) between middle and higher altitudes. The species in lower and the higher altitude (2901-3100 masl) showed higher dissimilarity (45% dissimilarity (Figure 2). This indicates that 45% of species that occurred in lower altitudes differed from the species occurring at higher altitudes. This could be because of more variation in an altitude, aspect, temperate, slope, and type of disturbance (timber extraction and grazing) between these two zones. The places with neighboring altitudes will have more similar species compared to places with huge altitudinal differences.

Diversity Indices

The diversity of plant communities is vital for the continuity and survival of all levels of an organism in the ecosystem. To determine the diversity of different plant communities, Shannon diversity was calculated for the study area. The diversity of vegetation (tree, shrub, and herb) increased till mid-altitude and decreased as altitude raised making a hump shape pattern.

The results concur with the findings of Xu et al., (2017) conducted in the mountainous ecosystem in China, which states that the diversity becomes higher at lower elevation till mid-altitude and then reduces when reaches the highest altitude. The overall diversity at the lowest altitude was ($H’ = 2.230$), mid-altitude ($H’ = 2.810$), and highest altitude ($H’ =2.580$). This study's findings are further supported by the mid-domain hypothesis (Zapata et al., 2003; Bhadra et al., 2010) which states overlapping of species richness in the middle and decreases along the edge. Due to this overlapping of species, it acts like a transition zone (Xu et al., 2017).

The evenness and species diversity in the study area follow a hump-shaped pattern with the highest values at the middle (that is 0.760, 2.830 at the lower altitude, 0.860, 3.950 in the middle altitude and 0.860, 3.050 at the higher altitude respectively (Figure 5). This is because the middle zone is free of disturbances like timber extraction and grazing by yaks and cattle. The majority of plots in middle altitude faces SE, E, SW direction, which allows more duration of sunlight leading to the growth of a variety of plants. The research carried in India in natural forests (mixed conifer, fir, chirpine forest) about the tree response to forest fire also found a uni-model pattern of diversity and evenness (invasive species) after the disturbance (Parashar and Biswas, 2018). This is because of extreme cold at a higher altitude and high competition at a lower altitude, which reduces invasive species' growth in these two zones (Parashar and Biswas, 2018).
Regeneration

The regeneration in the areas decreased with an increase in altitude. The regeneration at the lowest altitude was 142,500 seedling/ha, and in mid-altitude with 156,500 seedling/ha, and at the highest altitude with 130,000 seedling/ha. The pattern of regeneration varied across the site. The regeneration was high at the lowest altitude and decreased with further increase in altitude, making a humped shape pattern. This is maybe because of a steep slope, shady aspect, low temperature, and precipitation in the area.

The lower altitude has lesser diversity and regeneration due to human intervention and logging activities. Subedi et al. (2018) in chirpine and blue pine forest of Nepal found that when the number of disturbances increases, the diversity, and regeneration of tree decreases in the blue pine forest. The logging activities in the present study area not only reduce species diversity but also disturbs soil nutrient content through surface runoff. Thus diversity and regeneration were lesser at lower altitudes as compared to the middle altitude. This is also because most of the plots at lower altitudes (2500-2700 masl) face the SW and NW aspect with steep slopes that receive less sunlight, due to which the regeneration was minimum.
The overall regeneration status in the study area was good since the number of seedlings found in every plot was higher than the number of saplings. This indicates that the area is reviving and gaining ecological regain after the forest fire. Though overall regeneration was good, species like Pinus wallichiana showed poor regeneration (2.267 abundance), which falls under the rare class of abundance distribution (Maia, 2014). The results are similar to Neeman (2014) findings who reported that about 90% of blue pine saplings, trees, and seedlings are burned during a forest fire. This could be because the bark of blue pine is thinner, which gets burned quickly compared to other species. As a result, the regeneration of blue pine was less compared to chirpine other pine after the forest fire. The highest regenerations were found in Phalaris aquatica with 495,000 seedlings/ha and then of Desmodium sp with 404,166.67 seedlings/ha. Similarly, the lowest regenerations were found in species of Pinus wallichiana with 64,384 seedlings/ha, followed by Indigofera dosua with 37,500 seedlings/ha, then Jasminum sp. with 50,000 seedlings/ha and finally Berberis asiatica with 37,500 seedlings/ha (Figure 4). Furthermore, Pearson correlation was conducted to determine the effect of altitude on regeneration. The result showed that the relationship was not significant \( r = -.255, p = .174 \) (Table 2). The reason is that with the increase in altitude, the temperature, moisture, precipitation, organic content, and soil heat decrease. As a result, the number of plants germinating is less at high altitudes. The regeneration at low altitudes is less due to human disturbances. Dangwal and Tajinder (2016) the regeneration and diversity showed a hump-shaped pattern where there is higher regeneration at the middle altitude compared to the lower and higher altitude. 

**Effect of Aspect and Slope on Regeneration of Vegetation**

Aspect plays important role in the growth and regeneration of plant communities. The regeneration assessment showed that the east-facing slope had higher diversity \( H' = 1.910 \) followed by southeast \( H' = 1.710 \), northeast \( H' = 1.650 \), and southwest \( H' = 1.630 \). The regeneration of seedling was found higher in the east (146,500 seedlings/ha), southeast (150,000 seedlings/ha), and northeast 140,000 seedlings/ha. The findings are similar to research carried out in the Northern Province of China where the east and northeast aspects had higher diversity and plant growth than other aspects (Zeng et al., 2014).

The slope had a negative correlation with regeneration i.e., regeneration decreases with increasing slope. The species diversity \( H' = 1.850 \), with 145,000 seeding’s/ha was found at gentle slope (0-30), \( H' = 1.730 \) and regeneration of 142,000 seeding/ha at medium slope (30-60) and \( H' = 1.710 \) with regeneration of 140,000 seedlings/ha at steep slope (61-90). Zeng et al (2014) reported that the steep slope has low diversity as compared to the gentle slope due to differences in solar radiation intensity. The study conducted in the north province of china concluded that the gentle slope with low solar radiation could hold a higher amount of soil water which increases plant diversity (Zeng et al., 2014).

**Soil Nutrient Content of Burned Forest**

The soil nutrient (nitrogen, carbon, and potassium) have a similar pattern like that of diversity with higher nutrient contain at the mid-altitude except phosphorous with V shape pattern of distribution. The lower altitude has nitrogen, carbon and potassium, phosphorus contain of .050 , .590, 316.330 ppm, 3.270 mg/l and .080 , .960, 512.500 ppm, 1.210 mg/l at mid-altitude and .070 , .084, 493 ppm, 5.710 mg/l at higher altitude (Figure 5 and Figure 6).

The lower nutrient content at higher altitudes could be because of the low decomposition rate. Similarly, anthropogenic activities at lower altitudes could be a reason behind low soil nutrient content. The research carried out at Taibai mountain in the temperate zone by Zhang et al. (2019) found that the carbon, nitrogen, and phosphorous were high in the middle altitude as compared to lower and higher altitude but it was conducted in unburned mixed forest with similar altitude ranges. Zhang et al. (2019) reported that the mid-altitude forms a transition zone where plants from both higher and lower altitudes can grow, which increases organic matter.
The soil nutrient content was high at the mid-altitude after forest fire which could be due to burned plant residues (Sahu et al., 2019). The Pearson correlation conducted concludes correlation between diversity indices with soil pH, carbon, and phosphorous (Table 2). The correlation between diversity indices and soil nutrient was positive, carbon \((r = .407, p = .026)\), potassium \((r = .365, p = .470)\), phosphorous \((r = .636, p < .01)\). Wiehl and Prober (2011) also reported a positive correlation of soil nutrients with diversity indices, which is similar to this study's result. The correlation between diversity indices and soil nutrients was significant except for nitrogen \((r = .152, p = .442)\). This could be attributed to nitrogen forms being volatile, and since the study was conducted 6 months after the fire, the nitrogen content could be easily affected by wind, evaporation, and rain. Kutiel and Naveh (1987), also found the nitrogen content decreased by 25% after the fire, and it decreased further with time.

The correlation between regeneration and soil nutrient was conducted which showed positive correlation between regeneration and carbon \((r = .341)\), potassium \((r = 1.70)\), phosphorous \((r = .363, p<.049)\) and nitrogen \((r = .316)\). The correlation between regeneration and soil nutrient is not significant, except phosphorus for \(p<.05\) (Table 2). The soil nutrients increase after the forest fire as the fire chemically converts the nutrients in dead plants and adds them to soil surfaces. High nutrient contains in the soil leads to the growth of more plants in the area. Verma et al., (2017) reported a positive trend after forest fire in nutrient availability as there are more available nutrients in the soil after a fire. A higher amount of phosphorous in the soil after the forest fires was found because soil absorbed more phosphorous through the process of sorption, compared to others soil nutrients like N,
C., and K (Turrión et al., 2010). Phosphorous is an important component in plant development at an early stage, including its roots and ATP formation. Plants grow well where there is more available phosphorus (Day & Ludeke, 1993).

CONCLUSION
Forest fire not only damages landscape and biodiversity but also changes the quality of the soil. Forest management after a forest fire is crucial for the revival of flora and fauna. The study can be used as baseline data for the forest manager to sustainably restore forest after a fire. The study reports findings on soil nutrient content and species to be planted for plantation after a fire. The results can be also incorporated in management plans for sustainable management and conservation of mountain blue pine forests. The burned forest can be restored within a short period if we plant fire-resistant species like Q. semicarpifolia, which have higher resistance to fire and re-sprouts faster after the fire.

This study provides knowledge on selecting the native fire-resistant species as indicated in the study, which in turn helps in managing forest sustainably and increasing the livelihood of people in the long term. Since the study was carried out in a small area in a short time (winter season), it is difficult to conclude that the area is dominated by shrubs with higher regeneration than native tree species. Since most of the plants grow in summer, it is important to study both seasons (summer and winter) so that species response to fire over two seasons can be either compared or combined to generate the precise result.

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