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RESEARCH ARTICLE

Growth assessment of *Borinda grossa* after natural regeneration

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Abstract: Mass flowering of Borinda grossa occurred in 2015 in the central region of Bhutan. To assess its growth after natural regeneration, a field study was carried out in the Busa community forest (CF) located in Sephu gewog, Wangduephodrang district. A total of 39 sample plots of quadrat size 10 × 10 m were laid out in a study site of 80 hectares using systematic sampling to carry out bamboo inventory. The soil samples were collected using composite soil sampling from all the plots. Sample plots were categorized into bamboo presence and absence plots. Growth of bamboo was assessed in association with environmental factors. Wilcoxon signed rank test was used to compare environmental parameters between bamboo presence and absence plots. The result indicated significant differences in elevation, canopy cover and soil electrical conductivity. The growth of bamboo by diameter, height and count was highest in the elevation range between 2700 to 2800 m under medium canopy cover of 20 to 40% in loamy-sandy soil. Maximum number of bamboos was found in north and west facing slopes.

Keywords: Bamboo growth parameter, busa community forest, environmental factors, growth assessment

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Introduction

Globally there are 1718 known bamboo species and approximately 128 genera (Stapleton, 1994). Bamboo resources are extensively known as essential minor forest products and there are 22 million hectares of land covered with bamboo forest (Ohrnberger and Goerrings, 1984). Bamboo belongs to the grass family (Poaceae) and has tree-like woody stem (Lobovikov *et al.,* 2007).

The natural regeneration of bamboo is constrained by factors such as wildlife depredation, anthropogenic activities and invasion of weeds (Gopakumar and Motwani, 2013). The quality of natural regeneration of bamboo is dependent on the abundance of seeds and absence of depredation by the animals (Li *et al*., 2013). In Bhutan, there are more than 30 species of bamboo of which 10 species grow in the temperate regions (Stapleton *et al.,* 1997). *Borinda grossa* (*Rhui, Baa, Shi*) is one among ten temperate bamboos that is economically significant to rural community. *B. grossa* is found at an altitudinal range of 2600 m to 3200 m above sea level in wet, temperate mixed forest (Noltie, 2000). The main use of this species is for roofing, mats for animal and making temporary shelters. It is also used for house constructions, fencing, baskets and ropes. In Bhutan, mass flowering and death of *B. grossa* was recorded in 2005 at Sakteng (Wangda *et al.,* 2011), and later in 2015. This occurred in the temperate region of the country, hampering communities who were mostly dependent on *B. grossa* for their source of income by selling various bamboo products (Ministry of Agriculture [MoA], 2008).

From the climatic standpoint, it is mentioned that bamboo can contribute in carbon sequestration to mitigate climate change (Terefe, Jian and Kunyong, 2019). Whereas, climate change can result in the decline of bamboo habitat which in turn can pose a threat to the species diversity (e.g., herbivores such as panda) (Booth *et ai.,* 2014). Despite its significance, both in terms of conservation and economy besides a guideline for resource assessment and management of *B. grossa* (2008), there is no scientific research conducted in the country.

To understand its ecology and development after mass flowering, research on this important bamboo species was felt necessary. It is important to assess the growth after the natural regeneration of bamboo from its economic and conservation aspect (Bystriakova, Kapos and Lysenko, 2003). The study focuses on (1) comparison of environmental variables between *B. grossa* presence and absence plots, and (2) assessing the growth of *B. grossa* after its natural regeneration.

Materials and methods

Study Area

The study was conducted in the Busa community forest (CF) located in the Sephu *gewog* (sub-district) under the Wangduephodrang district (Fig. 1). Sephu is located in north-eastern part of Wangduephodrang with altitude ranging from 2600 to 3500 m (Sharma *et al.*, 2016). This CF was chosen for the study after confirming a natural regeneration of *B. grossa* since its mass flowering in 2015. Also, the people of Sephu use *B. grossa* for various purposes making traditional handicrafts, for fencing and roofing. As per the community forest management plan manual (2011), there are 40 households in the Busa village with tree species consisting of young blue pine (*Pinus wallichiana*), spruce (*Picea spinulosa*) hemlock (*Tsuga dumosa*) and cool broadleaf species. The common wildlife seen are wild pig (*Sus scrofa*), monkeys (*Macaca assamensis*), barking deer (*Muntiacus muntjak*), Himalayan black bear (*Ursus thibetanus*), sambar (*Rusa unicolor*), leopard (*Panthera pardus*) and birds.

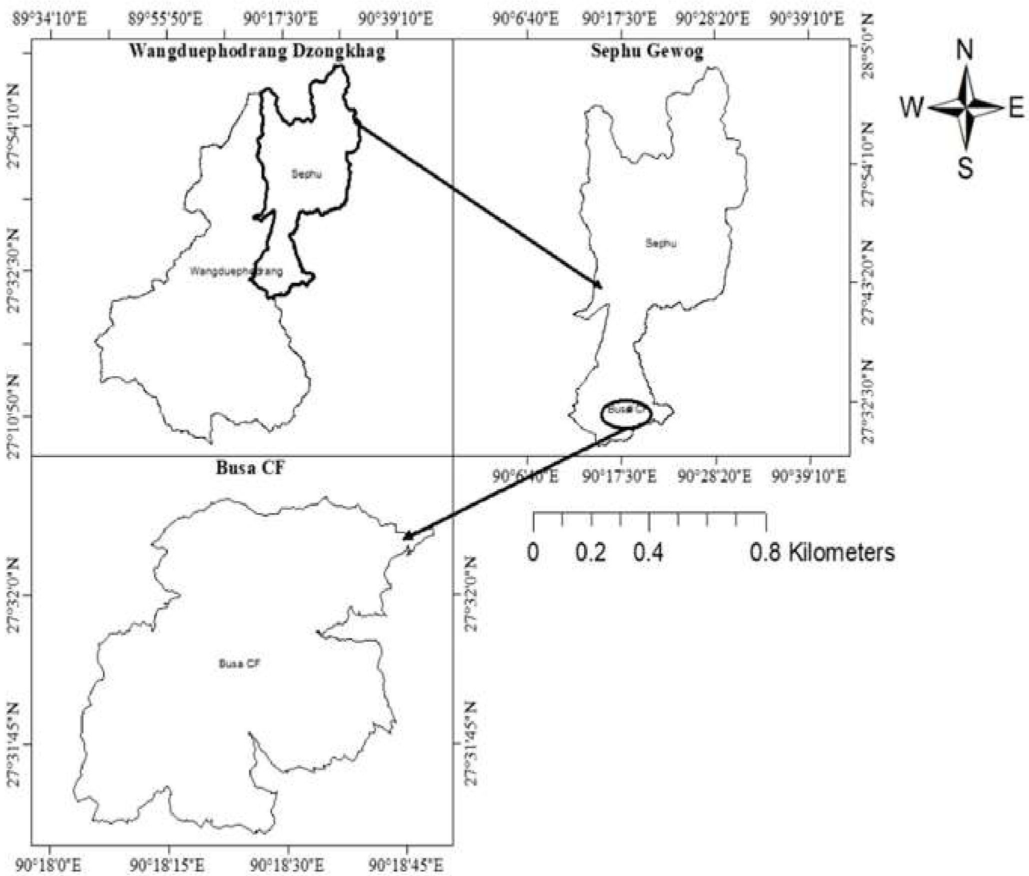

Fig 1. Study site

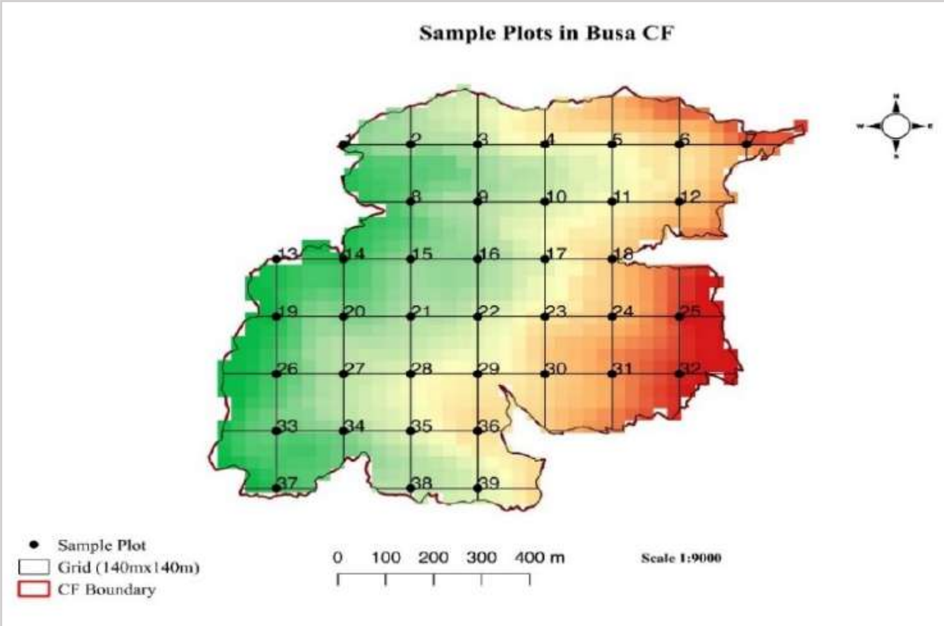

Fig 2. Sample plots layout

Sampling Design

Systematic sampling was carried out with a sampling intensity of 0.5%, as shown in Equation 1. The sampling plots were laid out using the grid system (Fig. 2) and adding sample points on the grid intersections. There were 39 plots within the area of 80 hectares of the Busa CF with a plot size of 10 × 10 m between the intervals of 140 m. The point shapefiles were created in ArcGIS 10.4 for uploading sample points to SW (Softwel) map which was used to locate the plots in the field. SW map is equipped with satellite image that shows real time user location and sample plots with Plot ID facilitating to track sample plot's location.

$$\text{Sampling Intensity} = \frac{\text{Plot size}}{\text{Grid size}} \text{ X } 100 \qquad \text{eq} - 1$$

$$\text{Sampling Intensity} = \frac{10 \text{ x } 10}{140 \text{ x } 140} \text{ X } 100 = 0.51\ \%$$

Data collection

Bamboo Inventory Data

A total of 39 sample plots were laid out using systematic sampling. The quadrat size of 10 × 10 m was set up with an interval of 140 m from plot to plot. Environmental factors such as elevation (measured by GPS handset), slope aspect (measured by Suunto compass) and visually estimated canopy cover (%) in each 10 × 10 m sample quadrat were recorded (Table 3). In each sample quadrat, the growth parameters of bamboo such as the height of the median culm, clump collar diameter, median culm collar diameter and the culm counts were measured. The height of the median culm was measured using a measuring tape, clump collar diameter and median culm collar diameter were measured using Vernier calliper, and the bamboo culm was counted in each occurrence plot (Table 4).

Soil data collection

A composite soil sampling method was used to collect soil samples. A total of 39 soil samples were collected with the depth of 0–30 cm. Soil samples were collected using a soil sampling auger. Five sub-samples of 10 x 10 m were collected from each of the 39 quadrats, with four from each corner and one from the centre. These sub-samples were then mixed thoroughly in a clean container.

Soil Analysis

As shown in table 1, analysis of physical soil properties soil bulk density (BD, g/cm^3), soil moisture content (SMC %), soil organic carbon (SOC %), soil organic matter (SOM %) and the analysis of chemical soil properties- essential macronutrients (NPK), soil pH, electrical conductivity (EC) were carried out in the laboratory using laboratory manual guide (Moktan, Wangmo and Rai, 2020) .

Table 1. Soil analysis methods

SI. No	Parameters	Methods	Instrument/apparatus
1	Soil organic carbon & soil organic matter	Loss on ignition method	Muffle furnace, oven, crucible, weighing machine
2	Soil moisture content	Gravimetric method	Oven
4	Soil bulk density	Cylindrical metal core method	Crucible, oven and muffle furnace
5	Available nitrogen	Loss on ignition method	
6	Available phosphorus	Bray no 2-extraction molybdenum blue method for available phosphorus	Spectrophotometer
7	Available potassium	Neutral 1 N ammonium acetate solution method	Flame photometer

Physical properties of soil

Soil texture: Soil texture was determined using hand feel method by referring hand feel soil texture chart (Thien, 1979).

Soil colour: It was determined using Munsell soil colour chart/book.

Soil Bulk Density (g/cm^3): Bulk density is calculated as the oven dried weight of a soil divided by the volume of the metal core container and measured in g/cm^3. The bulk density was determined using cylindrical core method (Hao *et al.*, 2006) as shown below:

$$\text{Bulk density} = \frac{\text{Oven dry weight}}{\text{Volume of soil}}$$

Soil moisture content (SMC %): Soil moisture content was measured using gravimetric method (Reynolds, 1970). First, the empty crucible weight and 5 g of soil samples were recorded. Secondly, it was placed inside the hot air oven at 105°C for 12 hours. Finally, the oven dried weight was recorded by computing the formula below:

$$\text{SMC\%} = \frac{\text{Initial Wt. of soil (g) - Wt. of oven dry soil (g)}}{\text{Initial weight of the soil (g)}} \text{ X } 100$$

Chemical properties of soil

*Soil pH***:** Soil pH of the samples was measured using digital pH meter. A buffer tablet was dissolved in 100 ml distilled water to make two buffer solutions with readings of 4 and 9, then the pH meter was standardized with buffer solutions (Pansu and Gautheyrou, 2006). The determination of soil reaction types was based on the pH range.

Soil Organic Matter (SOM %) and Soil Organic Carbon (SOC %): Soil organic matter and soil organic carbon were determined using Loss on Ignition (LOI) method (Hoogsteen *et al.*, 2015). LOI calculates % OM by comparing the weight of a sample before and after the soil has been ignited. The organic matter content was determined with the following equation:

$$\text{SOM\%} = \frac{\text{Wt. of oven dried soil (g) - Wt. of incinerated soil (g)}}{\text{Initial weight of the soil (g)}} \text{ X } 100$$

Since 58% of the mass of soil organic matter is normally comprised of soil organic carbon, the carbon content in the soil was estimated using the following formula:

$$\text{SOC \%} = \text{SOM \%} \times 0.58$$

Electrical conductivity **(EC):** Soil electrical conductivity (EC) was measured using an EC meter. The EC meter was calibrated with potassium chloride by immersing the electrode into the suspension to record the reading.

Data Analysis

The environmental variables between bamboo presence and absence plots were compared using Wilcoxon signed rank test $P \leq 0.05$. All the statistical tests were carried out in the statistical package for the social sciences (SPSS) programme.

Result and discussion

Descriptive analysis of the variables

The main environmental factors used for bamboo growth assessment were topographical factors, forest factors and soil parameters. As shown in Table 2, there were thirteen variables under three factors such as elevation (m), slope (%), aspect (slope directions), canopy cover (%), bulk density (BD, g/cm^3), soil moisture content (SMC %), soil organic matter (SOM %), soil organic carbon (SOC %), nitrogen content (%), available phosphorus parts per million (ppm), available potassium (ppm), pH and electrical conductivity (EC) measured in milli Siemens per metre (mS/m).

Soil properties Analysis

Physical soil properties included soil texture, soil colour and soil type. The soil type in the Busa CF varies from clay loam, sandy clay loam, sandy loamy soil, loamy sandy soil, loamy soil, silty loam to silt. Mostly, soil samples were loamy sandy soil with dark reddish colour. The reddish colour of the soil is may be attributed to the presence iron oxide, indicating a well-drained soil (Osunade, 1992) .

The pH of soil samples varied from neutral to moderately acidic reaction. The majority of samples were moderately acidic with the pH range 5.2 to 6.0. Sofiah *et al.*, (2018) reported that bamboo grows well in slightly acidic soil condition and pH acts as the indicator of soil

Table 2. Environmental factors

Environmental Factors	Variables	Categories
	Elevation (m)	Measured values divided into <2600, 2600-2700, 2700-2800, >2900
Topographical Factors	Slope (%)	Measured values divided into <10, 20-40, 50-70, >70
	Aspect (slope directions)	1-North, 2-Northeast, 3-East, 4-Southeast, 5-South, 6-Southwest, 7-West, 8-Northwest
Forest Factors	Canopy Cover (%)	Low (0-10), Medium (20-40), High (> 40-60)
		BD (g/cm^3)
Soil parameters		SOM (%)
		SMC (%)
		SOC (%)
	Nitrogen content (%)	
	Phosphorus (ppm)	
	Potassium (ppm)	
	pH	
	EC (mS/m)	

health for plant growth. The pH of a soil is important as it affects nutrient availability for plant growth (Johnston *et al*., 2014).

The bulk density of soil samples ranged from 0.22 to 1.07 g/cm^3. Soil moisture content ranged from 0.60 to 8.23%. Soil organic matter ranged from 0.05 to 7.41%. Soil organic carbon ranged from 0.09 to 5.09%. Nitrogen content in the study site was found to vary from 0.00 to 0.37%. Available phosphorus ranged from 1.02 to 3.89 parts per million (ppm) and available potassium ranged from 1.8 to 10 ppm. Soil electrical conductivity ranged from 4.16 to 35.6 mS/m.

Comparison of environmental factors between the bamboo presence and absence plots

The environmental factors between the bamboo presence and absence plots were compared using Wilcoxon signed rank test (Table 3) which revealed that the canopy cover in presence plot (Mdn = 20, SD = 18.34) is significantly lower than in absence plot (Mdn = 40, SD = 5.27, Z =-2.452, p = .014). The elevation was significantly lower in the presence plot (Mdn = 2721.5, SD = 64.51) than in the absence plot (Mdn = 2806, SD = 48.95, Z = -2.66, p = .007). Similarly, soil electrical conductivity was significantly lower in presence plot (Mdn = 9.85, SD = 3.55) than in absence plot (Mdn = 14.57, SD = 8.36, Z = -2.666, p = .007).

The maximum culm count (n=594) for *B. grossa* occurred under the canopy cover of 20-40% (Fig. 4), indicating this range as a better growing condition in the study area which differed from the report of Jihua (1991), where the high density of seedlings occurred under the canopy cover of 41 to 60 %. Regarding elevation, the bamboo culm counts (n=373) suggest that the elevation range is best suited between 2700 to 2800 m (Fig. 3) concurring with the report of Li *et al.,* (2013) in that the high density of seedling was found between the elevation from 2800 to 3000 m. For the growth of *B. grossa* the EC should be equal to or lower than 9.85 mS/m which is quite similar to the EC value (9.6 mS/m) of *Brassica campestris* L. ssp. *Chinensis* (Ding *et al.*, 2018). However, the increase in EC value (from 2 to 6 mS/m) resulted in linear decrease of fresh weight of tomato leaves, stems, and fruits according to Elia *et al*., 2000. This shows that EC value requirement for the growth of

Table 3. Comparison of variables between bamboo presence and absence plots using Wilcoxon signed ranked test

Environmental variables	Absence	Presence	Z	*P*
Slope (%)	65 ± 11.08	65 ± 20.84	-1.053b	0.292436
Elevation (m)	2806 ± 48.95	2721.5 ± 64.51	-2.666c	**0.007686**
Aspect	6 ± 2.6	6 ± 2.68	-.105b	0.916282
Canopy cover (%)	40 ± 5.27	20 ± 18.34	-2.452c	**0.01419**
Bulk density (g/cm3)	0.64272 ± 0.2	0.59 ± 0.19	-1.481b	0.138641
Soil Moisture Content (%)	3.73 ± 1.4	3.73 ± 1.51	-.140b	0.888638
Soil Organic Matter (%)	1.67 ± 2.01	1.35 ± 0.75	-1.599c	0.109745
Soil Organic Carbon (%)	2.84 ± 1.33	2.32 ± 1.29	-1.481c	0.138641
Nitrogen (%)	0.08 ± 0.1	0.07 ± 0.04	-1.599c	0.109745
Phosphorous conc. (ppm)	1.33 ± 0.48	1.39 ± 0.54	-1.007c	0.313938
Potassium (ppm)	3.3 ± 2	4.05 ± 2.07	-1.779b	0.075304
pH	5.98 ± 0.37	6.02 ± 0.39	-.889b	0.374259
EC (mS/m)	14.57 ± 8.36	9.85 ± 3.55	-2.666c	**0.007686**

b. Based on positive rank; c. Based on negative rank

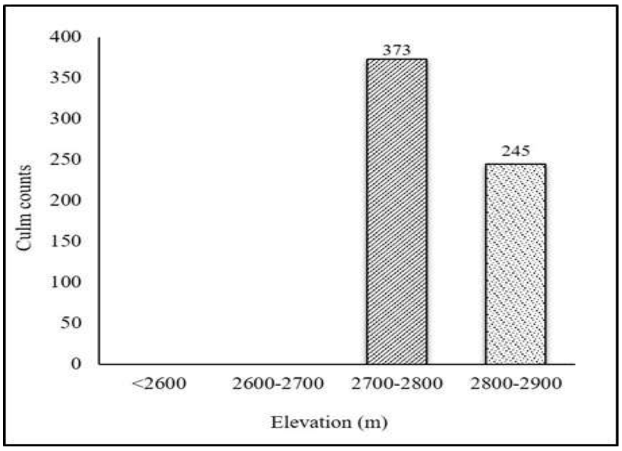

Fig 3. Bamboo culm counts in different elevation (m)

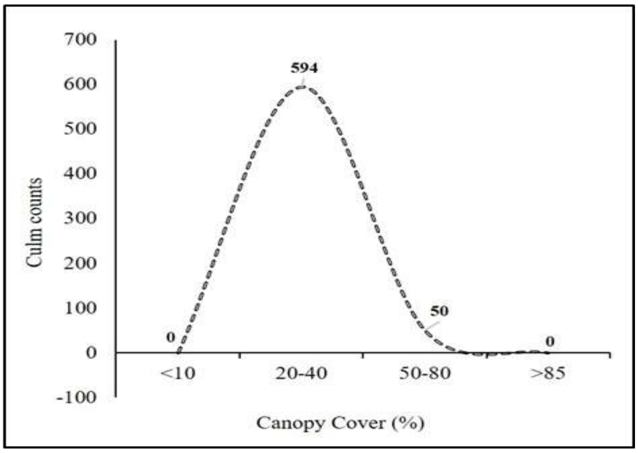

Fig 4. Bamboo culm counts in different canopy cover (%)

of different plant species varies and a common range cannot be prescribed.

Assessing the growth of B. grossa after natural regeneration

Distribution of bamboo was represented in only 9 plots. Information are all based on 9 plots as the remaining 30 plots did not fall under bamboo growing location.

Out of 9 plots, the maximum bamboo culm was found in plot 39 (n = 233) followed by plot 11 (n = 113) with the least number in plot 7 (n = 15) as shown in Table 4. Plot 39 had the highest soil bulk density (0.68 g/cm^3) and the least in plot 7 (0.12 g/cm^3). It was reported that soil bulk density higher than 1.6 g/cm^3 restricts the growth performance of plant (Chaudhari *et al.*, 2013) which is supported by the bigger median culm collar diameter (6.43 mm) and the median culm height of plot 39 (161.62 cm) compared with smaller median culm collar diameter (2.5 mm) and median culm height of plot 7 (63.25 cm).

Clump collar diameter (Fig. 5a), median bamboo culm height (Fig. 5b), median collar diameter (Fig. 5c), and culm count (Fig. 5d) were all greater in the north and west facing slopes than in the south and

Table 4. Bamboo presence plots with measured variables

Plot ID	Clump collar diameter (mm)	Culm counts (n)	Median culm collar diameter (mm)	Height of median culm (cm)
Plot 5	46.50	19.	4.50	95.00
Plot 6	69.85	97	3.58	100.46
Plot 7	38.75	15	2.50	63.25
Plot 11	102.59	113	5.55	138.23
Plot 12	64.00	28	2.56	53.11
Plot 25	91.69	31	4.87	93.12
Plot 31	124.57	58	7.42	170.42
Plot 34	135.50	24	5.75	133.75
Plot 39	161.76	233	6.43	161.62

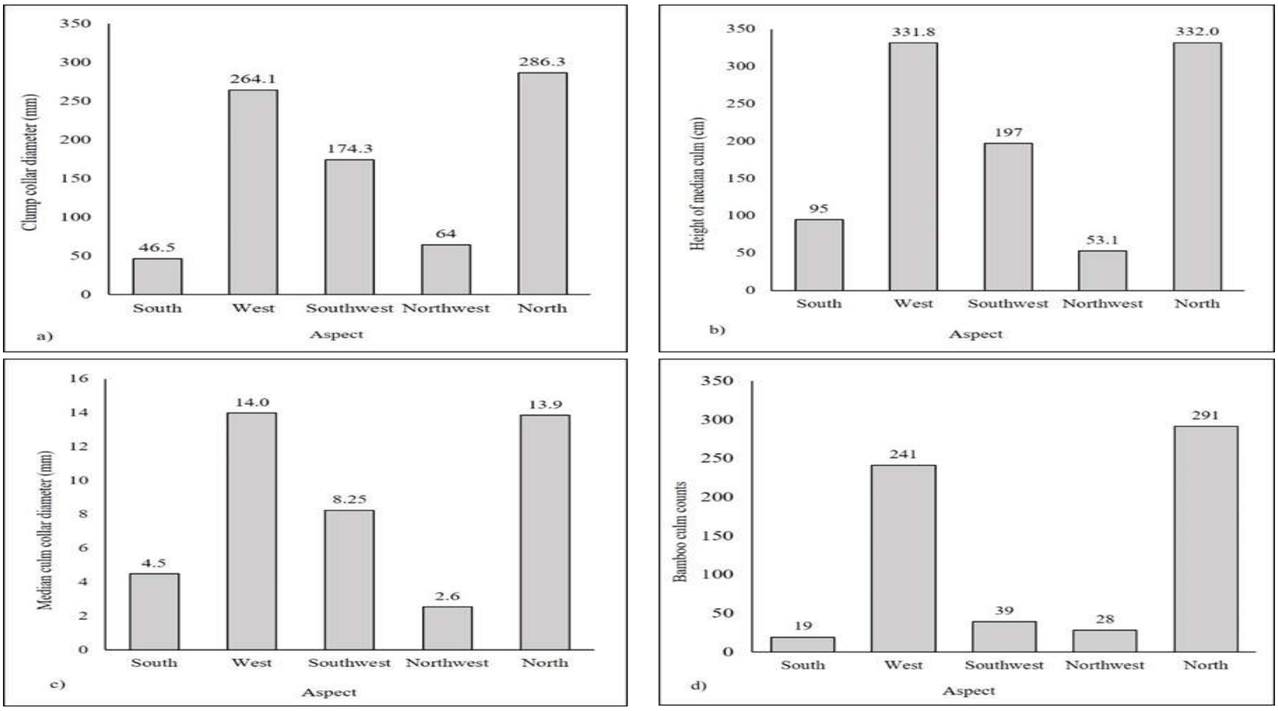

Fig 5. a) Clump collar diameter vs. aspect, b) Height of median culm vs. aspect, c) Median culm collar diameter vs. aspect, d) Bamboo culm counts vs. aspect

west facing slopes than in the south and northwest facing slopes. This corroborates with the results of habitat requirements (elevation 2700 to 2800 m) and moderate canopy cover (20 % to 40 %)) as shown in Fig.3 and Fig. 4 respectively and humid conditions (MoA, 2008).

Bamboo was found taller and more in number (Fig. 6) and larger in size in loamy sandy soil (Fig.7) which were similar to the report of Benjamin *et al.,* (2020) which states that bamboo thrives in loamy soil as it also combines with silt, sand and clay that help retaining moisture without waterlogging. On the other hand, sandy loam soils with a high concentration of sand, would easily dry and it is low in nutrients (Bruand *et al*., 2005) which supports the least growth result (Fig. 6 and Fig. 7).

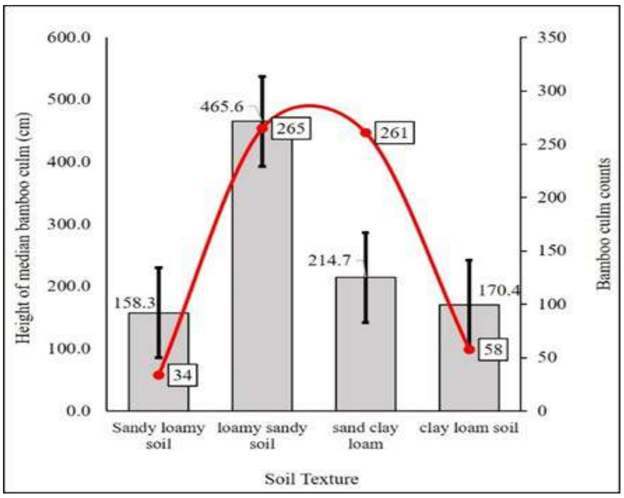

Fig 6. Height of median bamboo culm and culm counts in different soil texture.

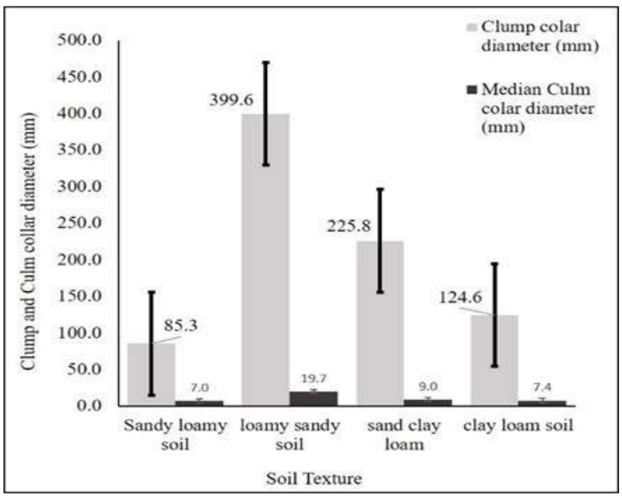

Fig 7. Clump collar diameter and median culm collar diameter in different soil texture

Conclusion

The study revealed that environmental factors such as elevation, canopy cover and soil electrical conductivity influence the habitat of *B. grossa.* The study and the literature suggest that the elevation range for the species is quite restrictive and narrow, thereby, the species ability to tolerate drastic change in climatic conditions such as global warming can lead to extirpation of the species.

The species appears to prefer some amount of shade and light, meaning that if canopy opening is more than 20 to 40%, the growth of *B. grossa* would be severely hampered. Hence, the management of Busa CF should opt for single tree selection silvicultural system rather than clear felling or group system. The present study provided soil electrical conductivity as an important indicator for bamboo growth wherein very high EC leads to inhibition of bamboo growth and low to medium EC promotes bamboo growth.

The growth of *B. grossa* was found better in the north facing aspect indicating that the species does not tolerate longer duration of solar insolation which was also indicated by the requirement of moderate canopy cover and higher elevation range. However, as any plant species, *B. grossa* also requires fertile soil such as loamy-sandy soil, with better nutrients and infiltration capacity.

During the field work it was observed that the upper and lower elevations of the study area were heavily grazed by cattle as well as by wild herbivores especially around the road and settlements. In addition, human activities such as harvesting of forest products were found to trample young seedlings disturbing the natural regeneration of bamboo. Such disturbances and interventions would have led to sporadic regeneration and growth of the species. To promote proper regeneration and growth, controlled grazing and well-planned extraction of forest products is recommended.

In this study, the parametric test could not be conducted due to small sample size which would otherwise provide a strong statistical result. Therefore, in conducting such research it is recommended that at least 30 sample points for each site type (e.g., presence site and absence site) should be considered so that comparison between the sites will be statistically sound.

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