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# Factors Affecting Natural Regeneration of Tropical Forests across a Precipitation Gradient in Myanmar

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**Abstract:** Natural regeneration is an essential component of forest dynamics and the recovery of ecosystem functions. Therefore, understanding regeneration status, and how abiotic and biotic factors affect it, is important for ecological studies. This study discovered different regeneration statuses of tropical forests in response to differences in rainfall in Myanmar, and the environmental and overstory factors that had the most influence on understory regeneration. Study sites were set up in regions with 625 to 2035 mm of annual rainfall, and ecological characteristics were measured. According to the results, natural regeneration increased with rainfall, showing a good regeneration status at all sites. Forests within a range of 1411–2035 mm of annual rainfall had a significantly higher density and species diversity at specific natural regeneration stages than those with 625–1029 mm. Not only abiotic but also overstory structure affected the natural regeneration of forests. However, not all factors influenced natural regeneration status. Overstory size distribution parameters did not show a significant influence on natural regeneration. Average annual rainfall (abiotic), as well as ecosystem complexity, density, species richness, and diversity (overstory), were found to be the most influential factors for the density and diversity of natural regeneration. The results of this study will support silviculture and the management of tropical forests.

**Keywords:** annual rainfall; density; diversity; ecosystem complexity; natural regeneration; overstory structure

## 1. Introduction

Natural regeneration is a key component for securing the sustainability of forests and forest dynamics [1–3] because it has the potential for the development of future generations, the storage of carbon, and it leads to the recovery of the ecosystem functions [4–6]. The research conducted in sub-tropical forests of India showed that an absence or a small number of seedlings and saplings of tree species in a forest stand indicates poor regeneration; on the other hand, a greater number of seedlings and saplings shows successful regeneration [7]. The maintenance of a forest stand with sufficient regeneration is the main target in conservation ecology [2].

Natural regeneration has also been used as a tool in the implementation of large-scale restoration programs in degraded forest areas [8,9], especially in the tropical regions [10,11]. When we consider species distribution and the diversity of forests in tropical ecology, natural regeneration should also be included in the assessment because it can help us to envisage the upcoming forest stand. Therefore,

understanding the mechanism and nature of natural regeneration facilitates the enhancement of natural regeneration, as well as the changes in future forest structure [12].

Natural regeneration could be affected by both abiotic (environment) and biotic (overstory structure) factors [1,2], and securing a sufficient regeneration of a forest is a great challenge in forest management. A reduction in precipitation, especially drought, can limit the regeneration potential and composition of plants because almost all species are very susceptible to environmental factors at the seedling stages [8,13,14]. Osem et al. [12] reported that species composition at regeneration stages varied in regions with an annual rainfall of 300 to 900 mm. Mok et al. [15] also estimated, by simulation analysis, that species diversity and composition at the regeneration stage could change in upcoming decades due to an increase in temperature and decrease in precipitation. On the other hand, changes in overstory structure, such as species distribution and canopy cover, could directly affect the diversity [2], survivorship [16–19], and composition [20,21] of natural regeneration of some species. Therefore, monitoring the changes in natural regeneration in response to different overstory structures and climatic regions has been essential in ecological studies.

Only a few studies have focused on natural regeneration ecology. Among them, some studies have addressed the effect of overstory structure on natural regeneration at relatively small scales in Acadian forests [22], disturbed forests [23], and Amazonian forests [24]. Although the factors driving the structure and composition of natural regeneration have been studied by modeling approaches [9], there is also no convincing evidence to date relating to which overstory factors have a stronger relationship with natural regeneration than others, especially in Asian regions. Beside this, the regeneration-rainfall gradient hypothesis has—to our knowledge—only been tested in a few studies. The way in which abiotic and biotic factors affect the structure and composition of natural regeneration by actual field surveys is still not well understood [25,26].

This study focused on cause-and-effect relationships between environmental factors and natural regeneration, and those between overstory structure and natural regeneration of tropical forests in Myanmar. So, the research questions were set up as: (1) Do species diversity, richness, density, and status of natural regeneration vary across a precipitation gradient in Myanmar? (2) Do environmental and overstory structural factors affect the status of natural regeneration? and (3) If so, how do these factors vary?

The objectives of the study were:

- (1) To observe the natural regeneration status of tropical forests in Myanmar across a precipitation gradient;
- (2) To extrapolate the effects of the environmental factors and overstory structure on natural regeneration of tropical forests; and
- (3) To identify the most influential environmental and overstory structure factors on natural regeneration of tropical forests.

## 2. Materials and Methods

### 2.1. Study Area

Study sites were located in tropical deciduous forests of Myanmar, having an average annual rainfall of 625 to 2035 mm (Figure 1).

The climate of the study sites is a tropical savanna climate. The climatic index of the study sites calculated by De Martonne's method [27–29] was 55.00 in site 1, 43.24 in site 2, 37.93 in site 3, 30.18 in site 4, 23.61 in site 5, and 16.80 in site 6. De Martonne's method is widely used to quantify aridity [29]. The higher the index value, the lower the aridity found. Generally, a value below 20 was considered arid or semi-arid, and more specifically, the upper limit for semi-arid was defined as 22.5, and for arid, it was set at 7.5 [28,30]. The average annual rainfall of sites 1 to 6 was 2035 mm, 1600 mm, 1411 mm, 1029 mm, 843 mm, and 625 mm, respectively, during 1982–2014 (Department of Meteorology and

Hydrology of Myanmar). The soil texture found in the study sites was sandy loam to sandy soil. The climatic variables of the study sites are shown in Table 1.

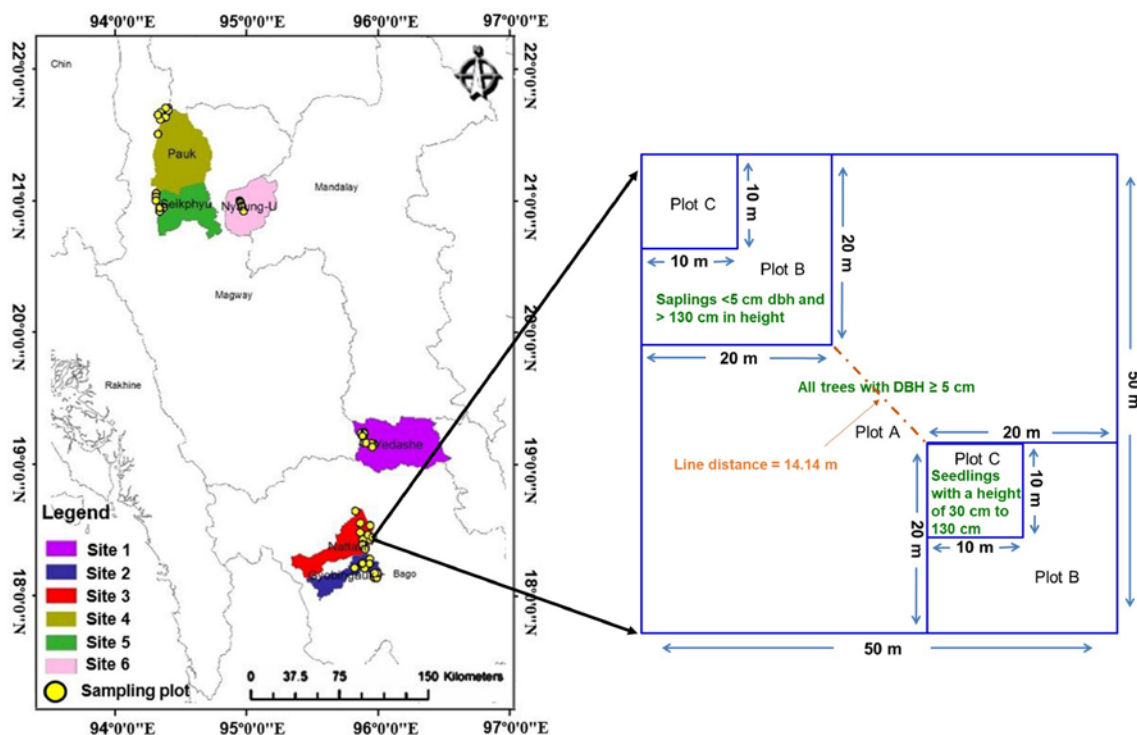


Figure 1. Location map of the study areas, and layout of sampling plots.

Table 1. Climatic variables of the study sites.

Site	Latitude (N)/Longitude (E)	Avg. R (mm)	Max. R (mm)	Min. R (mm)	Avg. T (°C)	Max. T (°C)	Min. T (°C)	R dif (mm)	T dif (°C)
Site 1	19°15'–19°23' / 95°51'–95°58'	2035	476	3	27.0	38.1	15.3	473	22.8
Site 2	18°15'–18°24' / 95°49'–95°59'	1600	323	1	27.0	38.7	15.4	322	23.3
Site 3	18°28'–18°46' / 95°49'–95°57'	1411	290	1	27.2	38.8	15.6	289	23.2
Site 4	21°38'–21°49' / 94°19'–94°24'	1029	200	1	24.1	34.6	12.4	199	22.2
Site 5	21°02'–21°11' / 94°18'–94°22'	843	191	0	25.7	36.2	14.0	191	22.2
Site 6	21°06'–21°07' / 94°56'–94°57'	625	124	0	27.2	39.5	14.5	124	25.0

Avg. R: mean annual rainfall; Max. R: maximum monthly rainfall; Min. R: minimum monthly rainfall; Avg. T: mean annual temperature; Max. T: maximum temperature; Min. T: minimum temperature; R dif: the difference between the highest rainfall and the lowest rainfall of the year; T dif: the difference between the highest and the lowest temperature of a year.

The forests at sites 1–5 were a heterogeneous forest type, and that at site 6 was a homogeneous forest type. The study sites are protected as 'reserve forests'. A diversity in species composition, as well as a variety of forest structural patterns, were dispersed for their representative sites. Economically important species were included in the forests, and the forests were managed by the 'Myanmar selection system' on a sustainable basis. However, illegal logging of trees and grazing were occurring in some places, and such areas were excluded from the survey for the collection of data. *Xylia dolabriformis* Benth. was one of the most dominant species at sites 1–5 [31], although it was not found at site 6. The dominant species at site 1 were *Xylia dolabriformis*, *Tectona grandis* L.f., *Protium serratum* (Wall. ex Colebr.) Engl., *Mitragyna rotundifolia* (Roxb.) Kuntze, and *Lagerstroemia tomentosa* C. Presl, whereas those at site 2 were *X. dolabriformis*, *Cordia grandis* Roxb., *M. rotundifolia*, *Dalbergia cultrate* var. *cultrata*., and *Lannea grandis* (Dennst.) Engl. At site 3, the dominant species were *X. dolabriformis*, *Terminalia tomentosa* Willd., *M. rotundifolia*, *Tectona grandis*, and *P. serrata*. The dominant species at site 4 were *X. dolabriformis*, *Pentacme siamensis* (Miq.) Kurz, *Terminalia tomentosa*, *Shorea oblongifolia* Thw., and *Dipterocarpus tuberculatus* Roxb., and those at site 5 were *Tectona hamiltoniana* Wall., *X. dolabriformis*, *Terminalia*

*oliveri* Brandis, *T. tomentosa*, and *Anogeissus acuminata* (Roxb. ex Candolle) Guillemain et al. [31]. At site 6, the dominant species were *Morinda tinctorial* Roxb., *Acacia leucophloea* (Roxb.) Willd, *Rhus paniculate* Wall., *Terminalia oliveri*, and *Acacia catechu* (L.f.) Willd.

## 2.2. Sampling Procedures

Plots for the regeneration survey were laid out within the sample plot (50 m × 50 m) in which the overstory structure survey was carried out. The sample plots were established using the stratified random sampling method. The stratification of forests was performed based on the appearance of tree density, coverage, and the occurrence of minor disturbance. Then, the sample plots were placed randomly in each stratum. All trees greater than 5 cm in diameter at breast height (dbh) in the 50 m × 50 m sample plots were recorded as the overstory structure in this study. Some of the overstory structure data were obtained from our former published research, Khaine et al. [31], because the same study sites were used. Within each sample plot (50 m × 50 m), two subsample plots of 20 m × 20 m, i.e., 400 m<sup>2</sup> each, were laid out in diagonally opposite corners of the plot (see Plot B in Figure 1). The diagonal distance between the nearest opposite corners of two subsample plots was 14.14 m. In total, the subsample plots covered 0.88 ha (22 plots) in site 1, 0.72 ha (18 plots) in site 2, 0.8 ha (20 plots) in site 3, 0.8 ha (20 plots) in site 4, 0.48 ha (12 plots) in site 5, and 0.48 ha (12 plots) in site 6. Saplings (<5 cm dbh and >130 cm in height) were counted in all the subsample plots. Therefore, a total of 104 plots, covering 4.16 ha, were used for the sapling survey.

For the seedling survey, two sub-subsample plots were set up in diagonally opposite corners of the subsample plot (20 m × 20 m) (see Plot C in Figure 1). The diagonal distance between the nearest opposite corners of two sub-subsample plots was 28.28 m. Each sub-subsample plot covered 100 m<sup>2</sup> (10 m × 10 m). Therefore, there were a total of 44 plots covering 0.44 ha in site 1, 36 plots covering 0.36 ha in site 2, 40 plots covering 0.40 ha in each of sites 3 and 4, and 24 plots covering 0.24 ha in each of sites 5 and 6. Seedlings with a height of 30 cm to 130 cm were counted in all sub-subsample plots. A total of 208 plots covering 2.08 ha were used for the seedling survey. The layout of sample plots is shown in Figure 1.

## 2.3. Data Analysis

Species identification of understory plants (natural regeneration) was done at the Plant Taxonomy and Seed Storage Division, Forest Research Institute of Myanmar. The density and the relative density of understory plants were explored on a hectare basis. Screening of dominant species at the natural regeneration stage was undertaken, based on the abundance of each species at each study site.

The Jackknife species richness [32], Shannon diversity index [33], and Simpson diversity index [34] of natural regeneration and over-story structure of forests were calculated as follows:

$$J = R + \{(n - 1)/n\}^k, \quad (1)$$

where,  $J$  = Jackknife species richness,  $R$  = total number of species,  $n$  = total number of plots, and  $k$  = number of unique species.

$$H' = \sum_{i=1}^s (Pi)(\ln Pi), \quad (2)$$

where,  $H'$  = Shannon's diversity index,  $s$  = number of species in the sample,  $Pi$  = proportion of the total sample belonging to the  $i$ th species, and  $\ln$  = natural logarithm.

$$E (\%) = 100 (H' / \ln H_{max}), \quad (3)$$

where,  $E$  = the Shannon evenness index in percentage,  $H'$  = Shannon's diversity index, and  $H_{max}$  = the number of total species.

$$D = \sum_{i=1}^s \{n_i (n_i - 1) / N (N - 1)\}, \quad (4)$$

where,  $(1 - D)$  = Simpson's index of diversity,  $n_i$  = number of individuals of species  $i$ ,  $s$  = number of species in the sample, and  $N$  = total number of individuals in the sample.

Duncan's multiple range test (DMRT) in an ANOVA analysis was used for a comparison of sites. Moreover, the transition of seedlings and saplings to mature stages was evaluated for each species, based on the ratio of their composition in a stand; the status of natural regeneration was determined based on these values [7,35], as shown in Table 2.

**Table 2.** Different regeneration statuses of a forest.

Sr. No.	Regeneration Status	Seedling (Se)	Sapling (Sa)	Compare to Mature
1	Good regeneration	ample	ample	Se > mature; Sa > mature
2	Fair regeneration	ample	conditional	Se > Sa; Sa ≤ mature
3	Poor regeneration	present absent	absent present	no Sa; but Se > mature no Se; but Sa > mature
4	No regeneration	absent	absent	only mature

As shown in the above table, 'good regeneration' is defined as the condition in which an ample or adequate number of seedlings and saplings contribute to the mature population, while 'fair regeneration' is defined as the condition in which there were a fair number of seedlings, but the percentage of saplings was either lower than or close to that of the mature trees. 'Poor regeneration' is the condition in which individuals were found at either the seedling or sapling stage only, in greater numbers than the mature trees. The fourth regeneration status is termed as 'no regeneration,' in which a species presented only at the mature stage and did not occur in either seedling or sapling stages. In this study, the regeneration status of a forest was determined based on a total population size of the forest, while the regeneration status of individual species was assessed on a population size of each species in a forest. The goodness of regeneration status could vary among species (taxonomic groups) even though they all showed a similar regeneration status in a forest. The reason for the variation among species is that the composition (density) of individual species in the total population of a forest differs among taxonomic groups. For example, although two species showed good regeneration statuses in a forest, a species which had a higher density than the other species could have a better regeneration potential in a comparison between the two species.

The spatial distribution of natural regeneration was conducted in this study because a complete characterization of regeneration should include a measure of spatial distribution [36,37]. Spatial distribution was computed using the formula used by Loetsch et al. [36] and Kershaw et al. [37]:

$$I = \delta^2 / \mu, \quad (5)$$

where,  $I$  = index of spatial distribution of a forest,  $\delta^2$  = population variation of the number of individuals per plot, and  $\mu$  = population mean of the number of individuals per plot.

Three parameters, namely, species diversity, species richness, and density of overstory structure of sites 1–5, were obtained from our previously published research conducted at the same sample plots [31]. These parameters for site 6, and other parameters of overstory structure for all sites, were calculated in this study. Size heterogeneity of forests at each study site was determined by the Gini coefficient [2,38]. Several studies have proved that this coefficient is very relevant for the evaluation of size heterogeneity of forest structure for most ecological questions [2,38,39]:

$$G = (\sum_{i=1}^n \sum_{j=1}^n |x_i - x_j|) / (2n^2 \mu), \quad (6)$$

where,  $G$  = Gini coefficient,  $x_i$  = the  $i$ th position of individual stems of a forest stand,  $x_j$  = the  $(i - 1)$ th position of individual stems of a forest stand,  $n$  = total individuals of a forest stand, and  $\mu$  = mean stem size of a forest stand.

Size distribution of overstory structure was evaluated by the skewness and kurtosis functions of the representative data (diameter, height, and basal area). The skewness and kurtosis functions have been found to be effective methods for the assessment of size distribution in multi-cohort stands [38,40]; therefore, the shape of the size distributions in this study was assessed using these functions.

SPSS statistical software (Version 24, International Business Machines Corporation (IBM), Armonk, NY, USA, 2016) was applied for all statistical analyses in this study. The effect of climate on natural regeneration was determined using Pearson correlation analysis at a 95% confidence interval. Climatic factors such as average annual rainfall (Avg. R), maximum rainfall (Max. R), average annual temperature (Avg. T), and temperature difference (T dif) were considered as independent variables, and the density per hectare, spatial distribution, and diversity of understory species in forests at all study sites as dependent variables. The correlation between climatic factors and understory structure was analyzed separately for seedlings and saplings. The values of spatial distribution used in the correlation analysis were site-level values.

For the effects of overstory structure parameters on natural regeneration, the most suitable biological factors were selected based on the relationships between response (natural regeneration) and explanatory (overstory) variables by means of Pearson correlation analysis in SPSS software. The overstory biological factors that were taken into consideration as factors influencing the density and richness of natural regeneration were: species richness, density, diversity, size heterogeneity, size distribution, and ecosystem complexity. The density was expressed on a per hectare basis, and the factors used in the correlation analysis were site-level values. Size heterogeneity (Gini coefficient) was determined based on tree diameter. Size distribution was assessed based on tree diameter, height, and basal area. Ecosystem complexity was calculated by the multiplication of the number of species, density, mean height, basal area, and times ( $2.5 \times 10^{-3}$ ) in each forest (0.1 ha basis) [41,42]. The response and explanatory variables are shown in Table 3.

A follow-up stepwise regression analysis was applied to investigate the combination effects of overstory structure on natural regeneration. Only the parameters that had a significant correlation with natural regeneration were used in the stepwise analysis. Eleven combinations of structure parameters were tested in this study.

**Table 3.** Variables of overstory structure and natural regeneration.

Description	Unit	Mean	SD	Range	
				Minimum	Maximum
<b>Response variables</b>					
Seedling density	trees	2026.33	726.95	770.00	2585.00
Sapling density	trees	1833.17	601.97	783.00	2261.00
Seedling richness	species	49.67	25.64	17.00	74.00
Sapling richness	species	49.17	25.89	17.00	74.00
Seedling diversity	-	1.76	0.29	1.44	2.04
Sapling diversity	-	2.25	0.37	1.76	2.63
<b>Explanatory variables</b>					
Over-story density	trees	306.83	51.59	229.00	368.00
Over-story richness	species	51.17	25.51	19.00	75.00
Over-story diversity	-	2.69	0.51	2.03	3.20
Size heterogeneity	cm	0.0061	0.0088	0.0019	0.024
Size distribution					
Skewness (D)	cm	0.96	0.78	0.18	2.27
Kurtosis (D)	cm	2.16	2.77	-0.21	6.25
Skewness (H)	m	0.70	0.55	0.17	1.40
Kurtosis (H)	m	1.24	1.99	-0.23	4.44
Skewness (BA)	m <sup>2</sup>	2.25	1.17	1.17	3.63
Kurtosis (BA)	m <sup>2</sup>	9.26	7.49	2.10	20.26
Ecosystem complexity	-	13.35	12.84	0.14	25.76

D: diameter; H: height; BA: basal area.

### 3. Results

#### 3.1. Seedling and Sapling Density

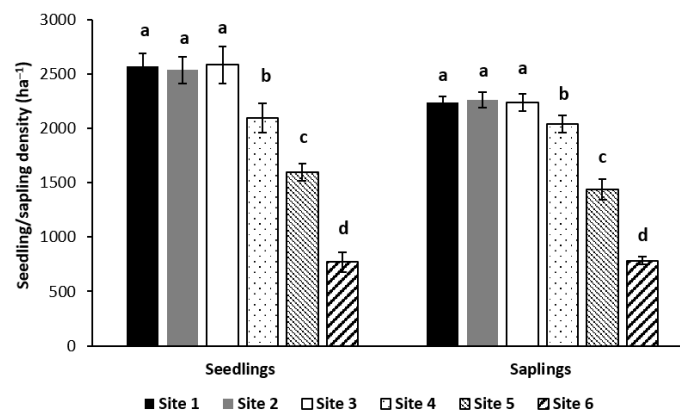
Natural regeneration of forests among study sites varied in density. In a comparison of study sites, both seedlings and saplings at sites 1–3 had significantly higher densities than at sites 4–6 ( $p < 0.05$ ). Densities of natural regeneration did not vary significantly among forests with 1411 to 2035 mm annual rainfall, whereas there was significant variation among forests with 625 to 1029 mm annual rainfall (95% confidence interval). The average seedling densities at sites 1–3 were 2575, 2536, and 2585 seedlings  $\text{ha}^{-1}$ , respectively, while the average seedling densities at sites 4–6 were 2097, 1596, and 771 seedlings  $\text{ha}^{-1}$ , respectively (Table 4). Similar results were found in the case of sapling density. Sapling densities did not show significant differences among sites 1 to 3 (2235, 2261, and 2241 saplings  $\text{ha}^{-1}$ , respectively), although the difference between sites 4, 5, and 6 was significant (2040, 1440, and 783 saplings  $\text{ha}^{-1}$ , respectively).

**Table 4.** Species richness of seedlings, saplings, and trees among six study sites.

Site	Seedling		Sapling		Tree	
	No. of Species	Jackknife Index	No. of Species	Jackknife Index	No. of Species	Jackknife Index
Site 1	74	74.83 <sup>a</sup>	74	74.95 <sup>a</sup>	75	75.83 <sup>a</sup>
Site 2	72	72.95 <sup>a</sup>	71	71.89 <sup>a</sup>	73	73.79 <sup>a</sup>
Site 3	67	67.93 <sup>a</sup>	68	69.00 <sup>a</sup>	69	69.73 <sup>a</sup>
Site 4	47	47.86 <sup>b</sup>	45	46.00 <sup>b</sup>	49	49.81 <sup>b</sup>
Site 5	21	21.92 <sup>c</sup>	20	21.00 <sup>c</sup>	22	23.00 <sup>c</sup>
Site 6	17	17.51 <sup>c</sup>	17	17.77 <sup>c</sup>	19	20.00 <sup>c</sup>

Different lowercase letters indicate the differences among the sites was significant by cluster analysis.

The highest seedling density was found at site 3, while the highest sapling density was found at site 2. Statistically, the seedling and sapling densities of forests in high rainfall regions were approximately three times higher than those of forests with 625 mm of annual rainfall (Figure 2). In addition, seedling densities were higher than sapling densities at the study sites, except at site 6. At site 6, where there was less rainfall, germination and survival at seedling stages may be difficult, but a species that has passed these stages may then be at an advantage and be able to survive in this area. Long-term continuous monitoring of the density of seedlings and saplings is required in each forest type because different ecosystems have functionally different regeneration dynamics, and thus, the canopy replacement in a future forest stand might differ or vary among different precipitation regions, even in a condition where the current regeneration status shows a similar species mixture with a similar density of a forest.



**Figure 2.** Seedling and sapling densities of forests at six study sites. Error bars show standard deviations. Different letters indicate significant differences among the sites by Duncan's multiple range tests at  $p < 0.05$ .

### 3.2. Species Richness and Diversity

The species richness values for seedling and sapling stages were lower than those for the mature stage at all the study sites and decreased with a decrease in annual rainfall (Table 4). Richness was highest at site 1 (high rainfall region), and the lowest at site 6 (low rainfall region). There were 74, 72, 67, 47, 21, and 17 seedling species at sites 1–6, respectively. At the sapling stage, there were 74, 71, 68, 47, 20, and 17 species at sites 1–6, respectively. Little difference between the number of species in seedling and sapling stages was found.

At seedling and sapling stages, the species diversity of forests differed between sites. Diversity tended to increase with rainfall, showing a significant increase at 1411 mm of annual rainfall ( $p < 0.05$ ). Shannon and Simpson indices of seedlings ranged from 1.44 to 2.04, and 0.79 to 0.90, respectively, and those of saplings ranged from 1.76 to 2.63, and 0.81 to 0.93, respectively (Table 5). The lowest species diversity at the regeneration stage was found at site 5. The difference between the sites was manifest at the 95% confidence interval.

**Table 5.** Different diversity indices of seedlings and saplings among six study sites.

Site	Seedling			Sapling		
	Shannon Diversity Index	Simpson Diversity Index	Shannon Evenness	Shannon Diversity Index	Simpson Diversity Index	Shannon Evenness
Site 1	2.01 ± 0.21 <sup>a</sup>	0.89 ± 0.03 <sup>a</sup>	96.99 ± 2.35 <sup>ab</sup>	2.49 ± 0.23 <sup>a</sup>	0.92 ± 0.02 <sup>ab</sup>	95.95 ± 1.92 <sup>a</sup>
Site 2	2.02 ± 0.15 <sup>a</sup>	0.90 ± 0.02 <sup>a</sup>	97.82 ± 1.59 <sup>a</sup>	2.58 ± 0.19 <sup>a</sup>	0.93 ± 0.02 <sup>a</sup>	96.39 ± 1.02 <sup>a</sup>
Site 3	2.04 ± 0.12 <sup>a</sup>	0.90 ± 0.02 <sup>a</sup>	97.61 ± 1.38 <sup>a</sup>	2.63 ± 0.08 <sup>a</sup>	0.93 ± 0.01 <sup>a</sup>	95.91 ± 1.41 <sup>a</sup>
Site 4	1.55 ± 0.20 <sup>b</sup>	0.81 ± 0.05 <sup>c</sup>	95.83 ± 3.91 <sup>bc</sup>	1.93 ± 0.35 <sup>c</sup>	0.84 ± 0.07 <sup>c</sup>	92.29 ± 5.75 <sup>bc</sup>
Site 5	1.44 ± 0.20 <sup>c</sup>	0.79 ± 0.06 <sup>d</sup>	94.89 ± 3.90 <sup>c</sup>	1.76 ± 0.27 <sup>d</sup>	0.81 ± 0.07 <sup>c</sup>	91.77 ± 5.80 <sup>c</sup>
Site 6	1.47 ± 0.11 <sup>bc</sup>	0.87 ± 0.04 <sup>b</sup>	94.61 ± 3.32 <sup>c</sup>	2.11 ± 0.08 <sup>b</sup>	0.89 ± 0.02 <sup>b</sup>	94.37 ± 2.40 <sup>ab</sup>

Different letters indicate significant differences among the sites by Duncan's multiple range tests at  $p < 0.05$ .

### 3.3. Species Composition at Regeneration Stages

The species composition of each forest type varied among the six study sites. For example, the most abundant species of the seedling and sapling layers at site 1 were *X. dolabriformis*, *T. grandis*, and *P. serrata*, whereas the most abundant species at site 2 were *X. dolabriformis*, *D. pentagyna*, and *C. grandis*. At site 3, *X. dolabriformis*, *Terminalia tomentosa*, and *M. rotundifolia* were the most abundant species, and at site 4, *Pentacme siamensis*, *Terminalia tomentosa*, and *X. dolabriformis* were the most abundant species. Whereas, the most abundant species at site 5 were *Tectona hamiltoniana*, *Terminalia tomentosa*, and *Terminalia oliveri*. The most important species at site 6 were completely different from the previous sites; the most abundant species at site 6 were *M. tinctoria*, *A. catechu*, and *A. leucophloea*. Table S1 shows the five most commonly occurring species at the six study sites.

### 3.4. Status of Natural Regeneration

Regeneration is a key process for the existence of species in a forest stand. The survival of seedlings or saplings determines successful regeneration, which is the single most successful step toward achieving long-term sustainability of forests [7,43,44]. The status of natural regeneration was determined depending on the transition between seedlings, saplings, and matures in each forest. The number of plants generally reduced in the transition from the seedling to sapling stages, and from the sapling to the mature stages, at all sites. Overall, the percentages of natural regeneration reaching mature stages were 7.0%, 7.7%, 6.7%, 7.8%, 7.5%, and 16.8% at sites 1–6, respectively. Thus, good natural regeneration occurred at all study sites, with sites 1 to 5 exhibiting better natural regeneration than site 6; this could also indicate that the proportion of plants in regeneration stages at sites 1–5 was much higher (two times) than at site 6. Table 6 shows the composition of seedlings, saplings, and mature trees of selected species at each study site.



Regeneration varied among species within a forest. As an example, at site 1, some species such as *X. dolabriformis* Benth., *Tectona grandis* Linn.f, and *Protium serrata* (Wall. Ex Colebr.) Engl. had a higher number of individuals at the seedling stage than at the sapling stage (Table 6), although some species such as *M. rotundifolia* (Roxb.) Kuntze and *L. tomentosa* C. Presl had a higher number of individuals at the sapling stage than at the seedling stage. *Azadirachta indica* A. Juss. was only present at the mature stage, and no regeneration of this species was found at site 1, although *X. dolabriformis* Benth., and *Tectona grandis* Linn.f displayed good regeneration. The number of seedlings and saplings of *X. dolabriformis* Benth. was 7.20 and 6.33 times, and the values of *Tectona grandis* Linn.f were 6.81 and 6.41 times, higher than the number of mature trees, respectively.

At site 2, most of the species exhibited good regeneration (e.g., *T. grandis* Linn.f, *X. dolabriformis* Benth., etc.) (Table 6). The number of seedlings and saplings of *T. grandis* Linn.f was 7.09 and 5.55 times, and those of *X. dolabriformis* Benth. was 6.86 and 6.21 times, higher than the number of mature trees, respectively. Nevertheless, no regeneration of *Rinorea bengalensis* (Wall.) Kuntze, as well as poor regeneration of *Lagerstroemia parviflora* Roxb., occurred at site 2. *Lagerstroemia parviflora* Roxb. had no saplings, although the number of seedlings was six times higher than the mature trees.

Similarly, some species such as *Pittosporum napaulensis* DC. (no seedlings and five times higher saplings than the mature) and *Cleidion speciflorum* Merr. (no saplings and five times higher seedlings than the mature) had poor natural regeneration, while *T. grandis* Linn.f (10.42 and 7.92 times higher seedlings and saplings, respectively, than the mature) and *X. dolabriformis* Benth. (6.85 and 6.96 times higher seedlings and saplings, respectively, than the mature) showed a good regeneration pattern at site 3 (Table 6). Likewise, *Morinda tinctoria* Roxb., *Acacia leucophloea* (Roxb.) Willd, *Rhus paniculata* Wall., and *Acacia catechu* (L.f.) Willd. exhibited good regeneration at site 6, whereas the regeneration of *Gardenia obtusifolia* Roxb. and *Lannea grandis* Engl. species did not occur at site 6 (Table 6). At site 6, *Morinda tinctoria* Roxb., *Acacia leucophloea* (Roxb.) Willd, *Rhus paniculata* Wall., and *Acacia catechu* (L.f.) Willd. had, respectively, 2.49, 4.80, 2.36, and 4.36 times higher seedlings, and 2.98, 3.15, 3.15, and 3.23 times higher saplings, than the mature trees.

In this study, the regeneration status of a forest was determined based on a total population size of the forest, while the regeneration status of individual species was assessed on a population size of each species in a forest. However, the goodness of the regeneration status could vary among species (taxonomic groups) even though they all showed a similar regeneration status in a forest. The reason for the variation among species is that the composition (density) of individual species in the total population of a forest differs among taxonomic groups. For example, although two species showed good regeneration statuses in a forest, a species which had a higher density than the other species could have a better regeneration potential in a comparison between the two species. For example, although both *X. dolabriformis* Benth., and *Protium serrata* (Wall. Ex Colebr.) Engl. had a good regeneration status, *X. dolabriformis* Benth. had a better regeneration potential than *Protium serrata* (Wall. Ex Colebr.) Engl. because *X. dolabriformis* Benth. had a higher density or composition than *Protium serrata* (Wall. Ex Colebr.) Engl. in a forest.

**Table 6.** Composition of seedlings, saplings, and mature trees of some species at six study sites.

Site	Species	Seedling (%)	Sapling (%)	Mature (%)	Total (%)
Site 1	<i>Xylia dolabriformis</i> Benth.	49.54	43.58	6.88	100
	<i>Tectona grandis</i> Linn.f	47.92	45.05	7.03	100
	<i>Protium serrata</i> (Wall. Ex Colebr.) Engl.	51.71	42.37	5.92	100
	<i>Mitragyna rotundifolia</i> (Roxb.) Kuntze	45.56	46.67	7.78	100
	<i>Lagerstroemia tomentosa</i> C. Presl	46.35	47.45	6.20	100
	<i>Azadirachta indica</i> A. Juss	0	0	100	100

Table 6. Cont.

Site	Species	Seedling (%)	Sapling (%)	Mature (%)	Total (%)
Site 2	<i>Xylia dolabriformis</i> Benth.	48.73	44.16	7.11	100
	<i>Mitragyna rotundifolia</i> (Roxb.) Kuntze	41.92	50.00	8.08	100
	<i>Tectona grandis</i> Linn.f	52.00	40.67	7.33	100
	<i>Lannea grandis</i> Engl.	48.28	43.10	8.62	100
	<i>Rinorea bengalensis</i> (Wall.) Kuntze	0	0	100	100
	<i>Lagerstroemia parviflora</i> Roxb.	85.71	0	14.29	100
Site 3	<i>Xylia dolabriformis</i> Benth.	46.23	47.01	6.75	100
	<i>Tectona grandis</i> Linn.f	53.88	40.95	5.17	100
	<i>Mitragyna rotundifolia</i> (Roxb.) Kuntze	47.84	45.32	6.83	100
	<i>Pittosporum nepaulensis</i> DC.	0	83.33	16.67	100
	<i>Stereospermum fimbriatum</i> Wall.	0	75.00	25.00	100
	<i>Cleidion speciflorum</i> Merr.	83.33	0	16.67	100
Site 4	<i>Xylia dolabriformis</i> Benth.	41.99	49.61	8.40	100
	<i>Pentacme siamensis</i> (Miq.) Kurz	45.06	47.13	7.80	100
	<i>Terminalia tomentosa</i> (Roxb.) Wight&Arn	46.82	46.10	7.08	100
	<i>Shorea oblongifolia</i> Thw.	44.97	47.62	7.41	100
	<i>Dipterocarpus tuberculatus</i> Roxb.	44.63	47.99	7.38	100
	<i>Holarrhena antidysenterica</i> (Linn.) Wall.	0	0	100	100
	<i>Premna latifolia</i> Roxb.	88.89	0	11.11	100
	<i>Diospyros burmanica</i> Kurz	0	83.33	16.67	100
Site 5	<i>Tectona hamiltoniana</i> Wall.	48.28	44.67	7.05	100
	<i>Terminalia oliveri</i> Brandis.	47.71	45.71	6.57	100
	<i>Terminalia tomentosa</i> (Roxb.) Wight&Arn	56.63	37.22	6.15	100
	<i>Anogeissus acuminata</i> (Roxb. Ex Candolle)	47.27	45.45	7.27	100
	<i>Pentacme siamensis</i> (Miq.) Kurz	72.73	0	27.27	100
	<i>Spondias pinnata</i> (L.f.) Kurz	0	0	100	100
Site 6	<i>Morinda tinctoria</i> Roxb.	38.49	46.05	15.46	100
	<i>Acacia leucophloea</i> (Roxb.) Willd	53.63	35.20	11.17	100
	<i>Rhus peniculata</i> Wall.	36.22	48.43	15.35	100
	<i>Acacia catechu</i> (L.f.) Willd.	50.79	37.57	11.64	100
	<i>Gardenia obtusifolia</i> Roxb.	0	0	100	100
	<i>Lannea grandis</i> Engl.	0	0	100	100

### 3.5. Spatial Distribution in Natural Regeneration of the Forest

Spatial distribution of natural regeneration at all study sites showed a regular distribution. Table 7 shows the spatial distribution of natural regeneration (seedling and sapling stages) at the study sites. A comparison of the sites showed that regeneration statuses in the seedling layers at sites 3 and 6, and in the sapling layers at site 5, were more clustered than at other sites. The index of spatial distribution at sites 1–6 was 0.05, 0.06, 0.11, 0.08, 0.04, and 0.11 at the seedling layers, and 0.06, 0.09, 0.11, 0.12, 0.25, and 0.07 at the sapling layers, respectively (Table 7).

Table 7. Spatial distribution of natural regeneration at seedling and sapling stages at the study sites.

Site	Index of Spatial Distribution at Seedling Stage	Index of Spatial Distribution at Sapling Stage
1	0.05	0.06
2	0.06	0.09
3	0.11	0.11
4	0.08	0.12
5	0.04	0.25
6	0.11	0.07

### 3.6. Effect of Climate on Natural Regeneration

The seedling and sapling densities were strongly affected by climatic factors such as the average annual rainfall, maximum rainfall, and the temperature difference ( $p < 0.01$ ). The seedling and sapling densities increased with average annual rainfall and maximum rainfall, indicating positive correlations between density, average annual rainfall, and maximum rainfall. Conversely, densities decreased when the temperature difference increased, showing a negative correlation between density and the

temperature difference (Table 8). Diversity was also affected by climatic factors in which average annual rainfall, maximum rainfall, and average annual temperature strongly affected the species diversity of forests (99% confidence interval). Spatial distribution did not show any correlation with climatic factors.

**Table 8.** Pearson correlation coefficients between forest growth and environmental factors.

Description	Avg. R	Max. R	Avg. T	T dif
Seedlings				
Density	0.819 **	0.747 **	0.141 *	−0.475 **
Shannon index	0.732 **	0.677 **	0.529 **	0.027
Simpson index	0.488 **	0.451 **	0.620 **	0.373 **
Shannon evenness	0.307 **	0.267 **	0.174 *	−0.037
Spatial distribution	−0.354	−0.421	0.239	0.649
Saplings				
Density	0.776 **	0.694 **	0.012	−0.538 **
Shannon index	0.620 **	0.560 **	0.631 **	0.250 *
Simpson index	0.485 **	0.440 **	0.605 **	0.360 **
Shannon evenness	0.342 **	0.316 **	0.393 **	0.204 *
Spatial distribution	−0.446	−0.397	−0.449	−0.560

\*\* and \* represent correlations are significant at  $p < 0.01$  and  $p < 0.05$  level, respectively; Avg. R: mean annual rainfall; Max. R: maximum rainfall; Avg. T: mean annual temperature; T dif: the difference between the highest temperature of the warmest month and the lowest temperature of the coldest month of the year.

### 3.7. Overstory Structure Parameters Influencing Natural Regeneration

Among overstory structure parameters, the overstory species richness, density, and ecosystem complexity were strongly correlated with density of natural regeneration, while the overstory density, richness, diversity, and ecosystem complexity were strongly correlated with species richness and diversity of natural regeneration (Table 9).

Parameters such as overstory size heterogeneity and distribution were not strongly correlated with density, richness, and diversity of natural regeneration. Therefore, these parameters should be excluded as predictors for the estimation of natural regeneration.

**Table 9.** The relationship between natural regeneration and overstory layers.

Description	SeD	SaD	SeR	SaR	SeDi	SaDi
Over-story density	0.797 *	0.819 *	0.919 **	0.911 *	0.828 *	0.780
Over-story richness	0.938 **	0.925 **	1.000 **	0.999 **	0.946 **	0.838 *
Over-story diversity	0.681	0.628	0.853 *	0.869 *	0.961 **	0.999 **
Size heterogeneity	0.351	0.304	0.459	0.465	0.430	0.342
Size distribution						
Skewness (D)	−0.516	−0.561	−0.215	−0.198	−0.046	0.190
Kurtosis (D)	−0.397	−0.438	−0.093	−0.077	0.035	0.234
Skewness (H)	−0.366	−0.416	−0.097	−0.083	0.019	0.169
Kurtosis (H)	−0.134	−0.180	0.115	0.128	0.176	0.264
Skewness (BA)	−0.153	−0.193	0.142	0.150	0.230	0.362
Kurtosis (BA)	0.105	0.055	0.381	0.390	0.456	0.536
Ecosystem complexity	0.860 *	0.812 *	0.953 **	0.961 **	0.995 **	0.929 **

\*\* and \* refer the relation are considered significant at  $p < 0.01$  and  $p < 0.05$ , respectively; SeD: seedling density; SaD: sapling density; SeR: seedling richness; SaR: sapling richness; SeDi: seedling diversity; SaDi: sapling diversity.

The combined effects of overstory species richness, density, diversity, and ecosystem complexity on natural regeneration status were clearly seen in this study, although the combined effects of these overstory parameters on natural regeneration of forests varied along with the included parameters (Table 10). Three factors, namely: overstory species richness, density, and ecosystem

complexity, were assessed using correlation tests, to identify interaction effects on the density of natural regeneration. Similarly, four factors, including overstory density, richness, diversity, and ecosystem complexity, were assessed using correlation tests, to identify interaction effects on the richness and diversity of natural regeneration.

Among four possible combinations for the density of natural regeneration, two combination effects, overstory density  $\times$  richness and overstory richness  $\times$  ecosystem complexity, significantly influenced the density of natural regeneration (Table 10). In the case of species richness and diversity of natural regeneration, the interaction effect of overstory density  $\times$  richness  $\times$  diversity  $\times$  ecosystem complexity was the most influential factor on the richness and diversity of natural regeneration among eleven possible combinations. Both species richness and diversity of natural regeneration were significantly influenced by the combination effects of overstory density, richness, diversity, and ecosystem complexity (Table 10).

**Table 10.** Effects of overstory structural parameters on natural regeneration.

Description	SeD	SaD	SeR	SaR	SeDi	SaDi
Ovs density $\times$ richness	0.911 *	0.863 *	1.000 **	0.999 **	0.908 *	0.702
Ovs density $\times$ EC	0.763	0.727	0.961 **	0.965 **	0.991 **	0.863
Ovs richness $\times$ EC	0.891 *	0.903 *	1.000 **	1.000 **	0.991 **	0.884 *
Ovs density $\times$ richness $\times$ EC	0.945	0.938	1.000 **	1.000 **	0.991 *	0.924
Ovs density $\times$ diversity			0.887 *	0.888 *	0.936 *	0.999 **
Ovs diversity $\times$ EC			0.926 *	0.936 *	0.995 **	1.000 **
Ovs richness $\times$ diversity			1.000 **	0.999 **	0.981 **	0.999 **
Ovs richness $\times$ diversity $\times$ EC			1.000 **	1.000 **	0.996 **	1.000 **
Ovs density $\times$ diversity $\times$ EC			0.983 *	0.980 *	0.995 **	1.000 **
Ovs density $\times$ richness $\times$ diversity			1.000 **	1.000 **	0.995 **	0.999 **
Ovs density $\times$ richness $\times$ diversity $\times$ EC			1.000 **	1.000 **	0.999 **	1.000 *

\*\* and \* refer that the effects are considered significant at  $p < 0.01$  and  $p < 0.05$ , respectively; SeD: seedling density; SaD: sapling density; SeR: seedling richness; SaR: sapling richness; SeDi: seedling diversity; SaDi: sapling diversity; OvS: Over-story; EC: ecosystem complexity.

#### 4. Discussion

The assessment of natural regeneration plays a vital role in sustainable forest management, because the regeneration stage is very important for the successful establishment of the mature community [2]. In this study, the densities of both seedlings and saplings at all sites were good enough for future regeneration from a management perspective, even though the density at lower rainfall regions (sites 4–6) was significantly lower than for the other sites.

Within the lower rainfall sites, the seedling and sapling densities of the forests with 1029 mm of annual rainfall and 843 mm of annual rainfall were 2.5 times and two times higher than those of the forests with 625 mm of annual rainfall, respectively. This finding supported the research by Galiano et al. [45] and Parada and Lusk [46], which explored how drought or reduced rainfall limited the survival of plants at the seedling stage. In comparison with other studies, the density of natural regeneration at our study sites was lower than that of semi-evergreen forests in Myanmar studied by Oo [47], and higher than that of the secondary forests in Vietnam studied by Woo et al. [48].

The richness of understory species in this study ranged from 17 species in the low rainfall region to 74 species in the high rainfall region, showing an increasing trend from low to high rainfall. Nonetheless, the species richness of forests at all sites in this study was lower than the richness found in semi-evergreen forests [47], although it was higher than that of dry forests in a semiarid region, where twelve regenerating species occurred [12].

Similar observations were made for species diversity. The results throughout the study sites showed an increasing trend with rainfall. Moreover, a noticeable difference (a significant increase) was found when annual rainfall changed from 625–1029 mm to 1411 mm. There was no evidence of a significance difference between forests with 625–1029 mm of annual rainfall.

Our study supported the statement developed by Mok et al. [15] that climatic variation was one of the factors that resulted in a shift in species niches, showing that species composition varied among forests in different rainfall regions. Moreover, this study showed that some species were distributed throughout a wide range of rainfall regions, whereas some species were adapted to a particular area. The natural regeneration of *T. grandis* and *X. dolabriformis* occurred throughout the regions where the annual rainfall was 843–2035 mm, showing that *X. dolabriformis* exhibited higher regeneration than *T. grandis* (Table S1). In addition, *T. grandis* occurred abundantly at sites 1–3 only, while *X. dolabriformis* occurred abundantly at sites 1–5. Therefore, the distribution range of *X. dolabriformis* was wider than that of *T. grandis*. However, those two species did not occur in a region with 625 mm of annual rainfall.

Furthermore, in a region with 625 mm of annual rainfall, a higher percentage of regeneration occurred for *M. tinctoria* and *A. catechu* species. However, those species were not observed in the high rainfall regions, particularly *M. tinctoria*, which was not observed in regions with 843–2035 mm of annual rainfall, while *A. catechu* did not occur in regions with 1029–2035 mm of annual rainfall. Therefore, low rainfall regions could be the preferred habitat of these species.

Most of the species at all sites had a good regeneration status, although a few species had a poor regeneration status or no natural regeneration. Numerically, 1.33% of total species at site 1, 1.37% at site 2, 2.04% at site 4, 4.55% at site 5, and 10.53% at site 6 revealed no natural regeneration. Moreover, 1.37% of total species at site 2, 4.35% at site 3, 4.08% at site 4, and 4.55% at site 5 demonstrated poor regeneration. The regeneration status found in this study was better than that of subtropical forests found by Khumbongmayum et al. [7], who reported that 2–16% of total species had no regeneration, and 13–29% had poor regeneration.

The spatial distribution of forests varied among sites, although the values did not differ significantly from each other. Within a range of 625–2035 mm of annual rainfall, the spatial distribution at all study sites was found to be regular, without showing any decreasing or increasing trends with annual rainfall.

Generally, natural regeneration largely depends on not only abiotic (temperature, rainfall, fertility, etc.), but also biotic (diversity, richness, composition, etc.) factors. In terms of abiotic factors, it is commonly accepted that the amount of rainfall is one of the essential factors for plant survival [49–51]. Our study supports that statement, in that the diversity and density of natural regeneration increased with rainfall. This study was also consistent with Osem et al. [12] findings that the density and richness increased linearly with rainfall in the regions where annual rainfall ranged from 300 to 900 mm.

This study highlighted that natural regeneration was more susceptible to the impact of rainfall in low rainfall regions than high rainfall regions, because the density of natural regeneration was significantly lower when annual rainfall decreased within the lower rainfall regions; however, a significant effect of rainfall on natural regeneration did not occur within high rainfall regions (Figure 2). Among climatic factors, average rainfall tended to be the most influential factor on the natural regeneration of forests, followed by maximum rainfall and average temperature.

In terms of biotic effects, understory species richness depends on overstory diversity [2,52]. Within a range of 625 to 2035 mm annual rainfall, the richer the overstory diversity of the forest, the richer the understory species recorded. The correlation of the diversity of mature trees to the diversities of seedlings and saplings was strong ( $r = 0.961, p \leq 0.01$  for seedlings, and  $r = 0.99, p \leq 0.01$  for saplings). Natural generation status was better in forests with a range of 843–2035 mm of annual rainfall than that in forests with 625 mm of annual rainfall.

Luo et al. [3] expressed that stand density was one of the factors that affected regeneration status. In this study, the overstory structure parameters that affected the natural regeneration of a forest were the overstory species richness, density, species diversity, and ecosystem complexity. Additionally, this study explored the combination of the influential overstory parameters that significantly affected species diversity and richness of natural regeneration, with the combination of two overstory factors, overstory density  $\times$  richness and overstory richness  $\times$  ecosystem complexity, showing significant effects.

The results from this study exhibited the variation of natural regeneration in response to different overstory structures across a rainfall gradient, which may indicate that there is potential for greater species diversity, richness, and density of upcoming forest stands with 1411–2035 mm of annual rainfall than those of forests with 625–1029 mm of annual rainfall. Although the density of upcoming forests with 1029 mm of annual rainfall is expected to be higher than those of forests with 625–843 mm of annual rainfall, the species diversity and richness of forests may remain the same between those regions, if there are no other disturbances. Overall, the possibility of a more diverse forest stand may occur in regions with 1411–2035 mm of annual rainfall than the other regions (625–1029 mm) in the future generation, and the overstory structure may control the natural regeneration of forests. The results from this study may form a basis for creating the next generation. A forest management approach, including silvicultural operations which can enhance natural regeneration such as canopy opening, climber cutting, weeding, enrichment planting, etc., may be given for maintaining forest biodiversity or creating the upcoming forest stands as a diverse forest with intended species.

## 5. Conclusions

The status of natural regeneration in tropical forests of Myanmar differed along rainfall gradients (a range of 625 to 2035 mm annual rainfall), although it demonstrated a good regeneration status, and regular species distributions, within that range of annual rainfall. The impact of climate was significant on species diversity, richness, and density of natural regeneration; an increasing trend occurred in regions with 625–2035 mm of annual rainfall. Forests with 1411 to 2035 mm of annual rainfall have a significantly higher density and species diversity at natural regeneration stages than those forests with an annual rainfall of 625 to 1029 mm. This study also revealed that species distributions, as well as the regeneration status of species at natural regeneration stages, varied from site to site, with a change of dominant species in the overstory structure. Both abiotic and biotic factors influenced the regeneration status. Among the environmental factors, average annual rainfall, followed by maximum rainfall and the temperature difference, strongly influenced the density of natural regeneration, although the average annual rainfall, followed by maximum rainfall and average annual temperature, influenced the diversity of natural regeneration. Nevertheless, the spatial distribution of natural regeneration could not be determined by the environmental factors. Biotic factors, such as: (1) the overstory density  $\times$  richness interaction, and (2) the overstory richness  $\times$  ecosystem complexity interaction, significantly influenced the density of natural regeneration. All combination effects of overstory density, richness, diversity, and ecosystem complexity significantly influenced species richness and the diversity of natural regeneration. It can be concluded that the natural regeneration of forests varied with both: (1) different rainfall gradients, and (2) overstory structure parameters, in terms of density, species richness, diversity, and ecosystem complexity. To control or conserve the natural regeneration of forests, amount of rainfall and overstory parameters should be taken into consideration in the forest management system.

**Supplementary Materials:** The following are available online at <http://www.mdpi.com/1999-4907/9/3/143/s1>, Table S1: Species mostly occurred at seedling and sapling layers among the study sites.

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