

Article

# Germination Phenological Response Identifies Flora Risk to Climate Change

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**Abstract:** Climate change is prevalent across the world and can have large influence on plant regeneration, recruitment, survival and diversity. Regeneration and recruitment are the key phases in the plant life cycle and these two aspects are related to survival, adaptation and distribution of species. This study thus aims to explore the effect of projected climate change on germination and establishment response of some timber tree species from the tropical/subtropical broad leaf forests of Nepal. Germination experiments were carried out under three different temperature regimes (20, 25 and 30 °C) and germination parameters identified from the experimental component were calibrated in the mechanistic model Tree and Climate Assessment—Germination and Establishment Module (TACA-GEM) that helped in identifying species vulnerability to climate change. The model outcome under varied climatic conditions helped in determining the species risk to projected climatic conditions. The model demonstrates that the studied species were able to increase germination under the projected climate change however, establishment consistently failed for most of the species across the hot tropical sites. This finding indicates that spatial vulnerability may limit recruitment in the future. The species-specific responses suggest that, in general, all three species (*Alnus nepalensis*, *Adina cordifolia*, and *Bombax ceiba*) exhibited enhanced germination and establishment in moderately warm and colder sites, indicating that these species may more likely shift their range towards the north in future. Thus, the general species response exhibited in this study may aid in regional climate change adaptation planning in the sector of forest conservation and management.

**Keywords:** regeneration; establishment; response; environmental change; adaptability

## 1. Introduction

Climate change is prevalent across the world [1–3] and is considered one of the most critical challenges of 21st century. The global temperature is expected to rise by 1.1–2.6 °C by the 2100s [4]. The Himalayan highlands however, seem to be warming at a rate several times greater than the global temperature average [5,6]. As warming is projected to be stronger in the Himalayan highlands, the rapid changes in environmental cues (temperature, water availability, soil moisture) may more likely affect plant physiology and phenology, changing the ecosystem and biodiversity of this region [7,8].

The effect of climate change is evident in Nepal and it is reported that the annual temperature in Nepal is rising by 0.41 °C per decade [9,10]. This is higher than the global average [11,12]. Climate change may adversely affect various sectors like forestry and agriculture, as well as impacting negatively on livelihoods and many other resources integral to the country's economy [13]. The National Adaptation Program of Action (NAPA) shows that Nepal is extremely vulnerable to climate change impacts because its economy heavily depends on natural resources, particularly water, soils, and forests [14]. Currently, more than 1.6 billion people depend on forests and forest products for their livelihood worldwide [15], and about 35% of the population in Nepal (1.45 million households) is

engaged in community forestry for their livelihoods [14]. Climate change is threatening community forestry, for which impacts are likely to be greater than for many other sectors [13]. This loss not only influences the functioning of the ecosystem and biodiversity but directly affects forest user groups [13] consequently affecting the economy of Nepal.

Climate change can have larger influence on plant regeneration, recruitment, survival and diversity [16,17] changing natural biological systems worldwide [4]. Germination marks the high-risk transition phase between seed and seedlings [18] in which environmental conditions strongly affect germination capacity [19]. This could in turn place species fecundity at risk [16]. Climate change may alter environmental signals like temperature, soil moisture, radiation and humidity that may prevent, delay or speed up the germination process, resulting germination phenological shift which consequently affects species composition and diversity [20]. However, the overall impact of climate change on plant regeneration has largely been neglected [21], and therefore studies on regeneration under climate change are crucial.

Forest composition generally depends on the regeneration of the species and their communities composing the forest in space and time [22]. Plant community structure and their distribution in many natural ecosystems are largely determined by species-specific traits and physiological behavior [23,24]. Phenological traits like flowering events, intensity, and airborne pollens are largely influenced by rainfall patterns, shown by the changed distribution of some woody and herbaceous species in the Spanish area of the Iberian Peninsula [25]. Germination (regeneration) and establishment (recruitment) represent two critical traits in the plant life cycle that are related to adaptation, community composition and distribution [26–28]. However, studies on these traits and future distribution of tree species have so far received only little attention [29,30]. Study of phenological traits can help in identifying the optimal species and varieties for plantation in changed climate conditions [31]. However, individual species phenological response modeling is crucial for the assessment of species resilience to climate change. Phenological models are widely used to study the impact of climate change on natural and managed ecosystems [31]. The Tree and Climate Assessment (TACA, [32–34]) and the Tree and Climate Assessment—Germination and Establishment Module (TACA-GEM [35]) mechanistic models have been largely used to assess the vulnerability of plant species to climate change, modeling the regeneration response. This model primarily utilizes species phenological parameters like growing degree days (GDD), base temperature, chilling requirement, frost, and drought to show the shift in species germination timing under projected climate conditions, that helps in the identification of vulnerable species [35,36].

The loss of tropical forest is reaching a critical level as it is shrinking by about 5% per decade, contributing to major biodiversity loss [15]. A study conducted by Thapa et al. [37] also indicates that climate change may adversely affect the lowlands and mid hills, consequently inviting major biodiversity loss in tropical and subtropical broad leaf forests by the 2050s and 2080s in several regions of Nepal [37]. Therefore, the current study explores the effect of projected climate change on regeneration and recruitment phenology of some timber tree species inhabiting tropical/subtropical broad leaf forests, using a forest vulnerability tool: the TACA-GEM mechanistic model [35,36]. Thus, the main objective of the study is to identify the resilient species that can adapt to projected climate change conditions by modeling species regeneration response.

## 2. Methods

### 2.1. Species and Study Area Selection

Seeds of the tropical/subtropical forest-inhabiting species *Adina cordifolia*, *Bombax cieba* and *Alnus nepalensis* were accessed from the forest seed suppliers. The species selection was based on the criterion that the species were classified as important indigenous timber species that have been recommended for afforestation programs in Nepal [38,39]. Descriptions of the species are provided in Table 1(a).

Six districts from mid-western Nepal with hot (Dumkauli, Rampur; past climate: ten-year average of maximum temperature  $\sim 30^{\circ}\text{C}$ ), warm (Nuwakot, Beni; past climate: ten-year average of maximum temperature  $\sim 25\text{--}26^{\circ}\text{C}$ ) and cool (Tamghas, Kanchikot; past climate: ten-year average of maximum temperature  $\sim 20\text{--}22^{\circ}\text{C}$ ) climatic conditions were considered for this study and details on the climate are provided in Table 1(b).

## 2.2. Germination Experiment

The experiment consisted of a randomized factorial design and was conducted in controlled seed germinator chambers. To identify the temperature and germination relationship, a germination test was carried out for each species following the guidelines provided by Rawal et al. [36]. Each species sample consisted of three replicates of 25 seeds germinated [40] at the average temperatures of  $30^{\circ}\text{C}$ ,  $25^{\circ}\text{C}$  and  $20^{\circ}\text{C}$ , respectively, under dark conditions. All the species were germinated in petri dish on top of filter paper (Whatman, 150 mm) except *Bombax cieba* seeds, which were germinated between the papers (grade germination paper) [41]. Moisture was maintained by adding 2 mL of distilled water every day [41]. Seeds were carefully observed for epicotyl hook emergence for each day.

**Table 1.** (a) Detailed description of the three selected species; (b) Name of place (where the weather stations were installed) followed by the station number, district name, yearly mean of maximum/minimum temperatures ( $T_{\text{max}}$  and  $T_{\text{min}}$ ,  $^{\circ}\text{C}$ ), yearly mean of total precipitation (PCP, mm) of the past climate (PC, ten years), and projected climatic condition (PCC, 2030–2060) of six stations in the mid-western region of Nepal used for Tree and Climate Assessment—Germination and Establishment Module (TACA-GEM) simulation.

(a)					
Species	Climatic Zone	Optimal Temp. Range ( $^{\circ}\text{C}$ )	Absolute Temp. Range ( $^{\circ}\text{C}$ )	Optimal Rainfall Range ( $\text{mm}/\text{yr}^{-1}$ )	Altitude Range (m)
<i>Adina cordifolia</i> (Karma) <sup>a,c</sup>	tropical wet/dry and wet	25–35	5–47	800–2000	0–800
<i>Alnusnepalensis</i> (Utis) <sup>a,b</sup>	tropical wet/dry, steppe or semiarid, subtropical humid, temperate, oceanic/continental, temperate with humid winters/dry winters	13–26	4–36	1000–2000	500–3000 <sup>b</sup>
<i>Bombaxceiba</i> (Simal) <sup>a</sup>	tropical wet/dry, steppe or semiarid	28–42	5–49	750–4000	0–1500

Source: <sup>a</sup> [42], <sup>b</sup> [43], <sup>c</sup> [44].

(b)									
Serial Number	District Name	Geographical Position	Altitude (m)	PC Average, 10 Years		PCC Average, 2030–2061			
				$T_{\text{min}}$	$T_{\text{max}}$	PCP	$T_{\text{min}}$	$T_{\text{max}}$	PCP
1	Beni (609) (Myagdi)	28°21' 83°34'	835	14.38	25.69	144.29	14.82	26.33	152.13
2	Dumkauli (706) (Nawalparasi)	27°41' 84°13'	154	18.67	30.64	208.68	19.77	31.51	192.53
3	Kanchikot (715) (Argakhachi)	27°56' 83°09'	1760	12.79	20.22	162.77	15.40	24.00	156.22
4	Nuwakot (1004) (Nuwakot)	27°55' 85°10'	1003	16.32	26.77	167.92	15.14	26.14	196.03
5	Rampur (902) (Chitwan)	27°37' 84°25'	256	17.95	30.98	161.07	18.95	31.06	178.55
6	Tamghas (725) (Gulmi)	28°04' 83°15'	1530	12.42	22.3	167.68	15.49	25.37	165.87

## 2.3. Statistical Analysis

The effect of temperature treatment on the success of species germination was tested using survival analysis following the methods of Rawal et al. [36] and Ranieri et al. [45]. Cox's proportional

hazard analysis [46] was performed to calculate the proportional hazard of temperature factors on germinated seeds using SPSS version20 [47]. For the purpose of analysis, germinated seeds were considered analogous to survival and seeds that did not germinate were considered equivalent to mortality [45].

To determine the optimum time and temperature needed for the highest germination percentage, germination percentage was analyzed as a dependent variable of GDD accumulation (GDD = Mean Temperature—Base Temperature × time in days) [36,48–50] using non-linear polynomial regression analysis [51]. For each species, the non-linear polynomial regression function derived was implemented in the mechanistic model.

#### 2.4. Mechanistic Model

The TACA-GEM (Mok et al., [35]) is a modified version of TACA [32–34] was used to model the species-specific germination and establishment responses for the assessment of species resiliency to climate change. The model analyses the influence of projected climate change on the ability of a tree species to regenerate and establish.

TACA-GEM primarily combines phenology with germination physiology which interacts with temperature, soil moisture, and frost, and simulates the species ability to regenerate and establish under climate-changed conditions. Species-specific germination parameters, GDD thresholds and GDD functions identified from the germination experiment plus the statistical analysis were implemented into the model. In detail, the model utilizes the germination experimental data where seeds germinated at a given GDD sum are regressed against GDD. The cumulative response function derived from the statistical analysis implemented in the model enables the germination to occur at variable rates and times of a year relying on the variable temperature and moisture throughout the year [36]. The use of a 365-day period (year) of variable temperature and moisture regimes allows for seeds to remain quiescent in periods that are unsuitable but allow for uniform or periodic germination as conditions permit throughout the year. The model then provides germination and establishment probability response outputs simulated for past and projected climatic conditions that helps in the assessment of species vulnerability and future abundance in light of projected climate change. This model can be simulated for 10 to 51 years of climate data. Details of the model can be found in the works of Rawal et al. [36], Mok et al. [35] and Nitschke and Innes [33]. Details on parameters for TACA-GEM input are provided in Table 2.

**Table 2.** Species specific germination parameters and climatic origin taken for the calibration of the TACA-GEM model.

Parameter	Species		
	<i>Adina cordifolia</i>	<i>Alnusnepalensis</i>	<i>Bombaxceiba</i>
<i>Geographic origin of seed</i>	27.1024, 85.5720	27.2593, 85.2930	26.55.3185.59.68
<i>Habitat</i>			
Soil texture	Sandy/clay <sup>6</sup>	Sandy <sup>5</sup>	Sandy loam <sup>9</sup>
Seedfall Julian date (days)	180 <sup>6</sup>	90 <sup>7</sup>	180 <sup>9</sup>
Rooting zone depth (m) <sup>1</sup>	0.10	0.10	0.10
Coarse fragment (%) <sup>1</sup>	0.30	0.10	0.30
<i>Probabilistic Germination Functions thresholds of polynomial regression for germination based on GDD</i>			
Minimum GDD threshold (days)	130	130	10
Maximum GDD threshold (days)	560	530	170
Minimum temperature (°C)	15	15	15
Maximum temperature (°C)	35	35 <sup>8</sup>	35
b0	−0.0415	−0.0353	−0.0004
b1	0.0004	0.0004	0.0017
b2	−5.7E-07	−5.3E-07	−1.8E-05
b3			4.6E-08

Table 2. Cont.

Parameter	Species		
	<i>Adina cordifolia</i>	<i>Almusnepalensis</i>	<i>Bombaxceiba</i>
<i>Other Germination Parameters</i>			
Germination moisture threshold (MPa)	−2	−2	−2
Physiological Base Temperature (°C)	5 <sup>2</sup>	5 <sup>2</sup>	5 <sup>2</sup>
<i>Establishment Parameters</i>			
GDD minimum	4000 <sup>3</sup>	3500 <sup>3</sup>	4500 <sup>3</sup>
GDD maximum	7000 <sup>3</sup>	6000 <sup>3</sup>	7000 <sup>3</sup>
Frost tolerance (0–1)	0 <sup>3,4</sup>	0.1 <sup>3,4</sup>	0.1 <sup>2,3,4</sup>
Frost season length (days)	0–10 <sup>3,6</sup>	30 <sup>3</sup>	30 <sup>3</sup>
Heat moisture index (dimensionless) <sup>4</sup>	47.5 <sup>2,3,4</sup>	34 <sup>2,3,4</sup>	50.66 <sup>3,4</sup>
Drought tolerance	0.30	0.55 <sup>3</sup>	0.30 <sup>2,4</sup>
Minimum temperature	0 <sup>2</sup>	−1 <sup>2</sup>	−3 <sup>2</sup>

GDD: growing degree days; <sup>1</sup> [35]; <sup>2</sup> [42]; <sup>3</sup> [52]; <sup>4</sup> [33]; <sup>5</sup> [43]; <sup>6</sup> [53]; <sup>7</sup> [54]; <sup>8</sup> [55]; <sup>9</sup> [56].

### 2.5. Climate Parameters and Scenarios

For the simulation of past (observed) climate (PC), the information from 10 reference years was input to TACA-GEM from each of the 6 districts (period 1989–1999 for all districts except for Nuwakot, 1988–1998). The climatic station number, name of place, and district are provided in Table 1(b). The climate data include the minimum and maximum temperature ( $T_{\min}$  and  $T_{\max}$ ), precipitation (PCP), and solar radiation accessed from the Department of Hydrology and Meteorology (DHM, Nepal).

For the projected climate simulation, scenarios from DHM has been used (Source: DHM, Department of Hydrology and Meteorology, Nepal). The average of the PRECIS-ECHAM5 and HadCM3 models (biased corrected) representing the A1B scenario (moderate climate change scenario) by the 2030s–2060s has been selected for model simulation. The PRECIS model (Regional Climate for Impact Studies) is one of the best dynamical downscaling tools. It was developed at the Met Office and Hadley Center, and is based on atmospheric component of the HadCM3 Global Climate Model. The base reference years used to build the projected climatic condition were from the years 1970–2000 (Nepal Climate data portal user manual, DHM, [www.dhm.gov.np/dpc](http://www.dhm.gov.np/dpc)). The past climate conditions and the projected climatic conditions are henceforth termed as PC and PCC, respectively.

Daily solar insolation (sunshine hours) data exists only for the station Pokhara airport in the mid-western region of Nepal, hence this daily solar insolation data has been used for the model simulation for all the sites selected. Solar insolation has been kept constant for the past and projected scenarios. Where the missing values exist for the climatic factors, the monthly means of the climatic variables  $T_{\min}/T_{\max}/R_{\text{mean}}$ /solar insolation were added. When monthly solar insolation values were missing, the monthly mean of previous or subsequent year was used for the interpolation. Daily solar insolation was converted to solar radiation for the model input.

## 3. Results

### 3.1. Climatic Condition of the Sites

Monthly mean of maximum and minimum temperatures of the PC and PCC show that Dumkauli and Rampur have similar climatic conditions with hot temperature conditions than the other sites (Table 1(b)). Climatic conditions were colder at Kanchikot and Tamghas under the past and projected climate, whereas the climatic conditions of other two sites Beni and Nuwakot were moderately warm. However, the temperature of Nuwakot is quite consistent, with no temperature increment from PC to PCC. The lowest rainfall condition was found in Beni under PC and PCC (Table 1(b)).

### 3.2. Experimental Germination Result

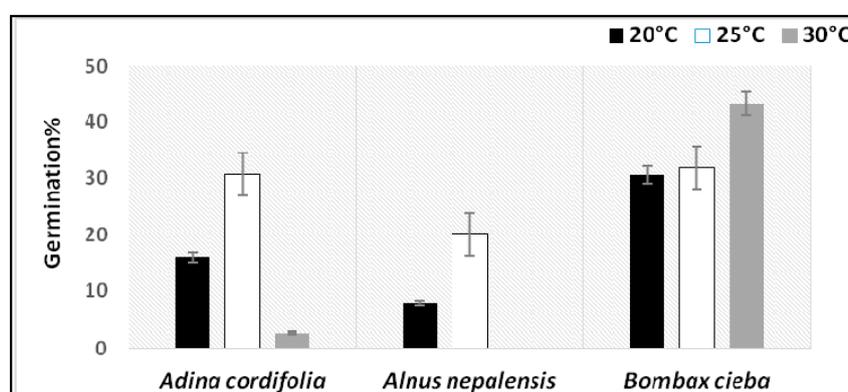
Species germination response to three different temperature regimes demonstrated that temperature had significant effect on the germination of *A. cordifolia* and *A. nepalensis* (Table 3).

For both of these species, germination was greater at the temperature condition of 25 °C. *Adina cordifolia* exhibited lower germination and *A. nepalensis* failed to germinate at 30 °C (Figure 1). Although the temperature difference was not significant, germination was higher for *B. cieba* at 30 °C (Table 3, Figure 1).

**Table 3.** Cox’s proportional hazard regression analysis on germination of three species indicating the significance temperature.

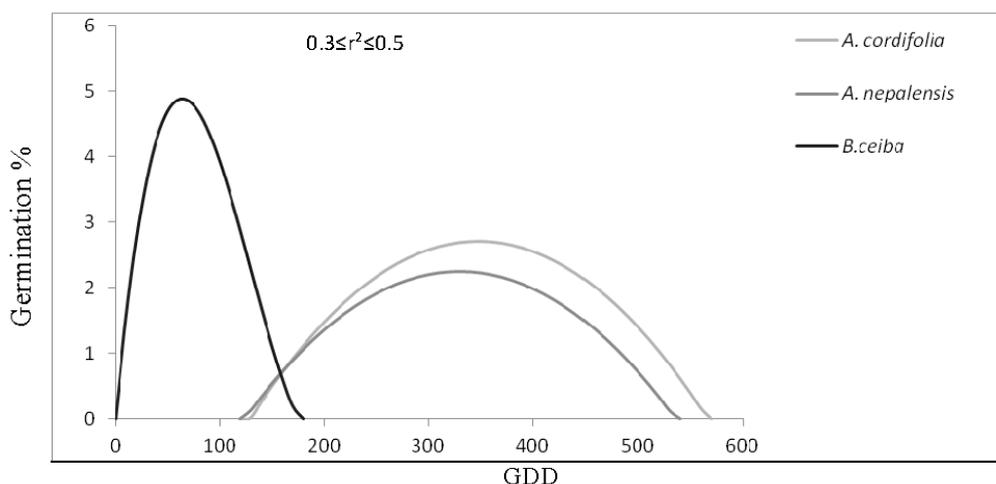
Species	Temperature	Wald
<i>Adina cordifolia</i>	* ( $p = 0.018$ )	8.004
<i>Alnusnepalensis</i>	* ( $p = 0.023$ )	7.506
<i>Bombaxcieba</i>	ns ( $p = 0.335$ )	2.189

\*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$ ; ns, not significant.



**Figure 1.** Germination percentage of three species under three different temperature regimes (20°C, 25°C and 30°C).

Non-linear regression modeling identified significant relationships between GDD requirements and the initiation and cessation of germination. The resultant models show that within the tested species, *B. ceiba* has narrow germination window with fewer GDDs required to achieve optimum germination. Again this species exhibited greater germination success than *A. cordifolia* and *A. nepalensis* (Figures 1 and 2).

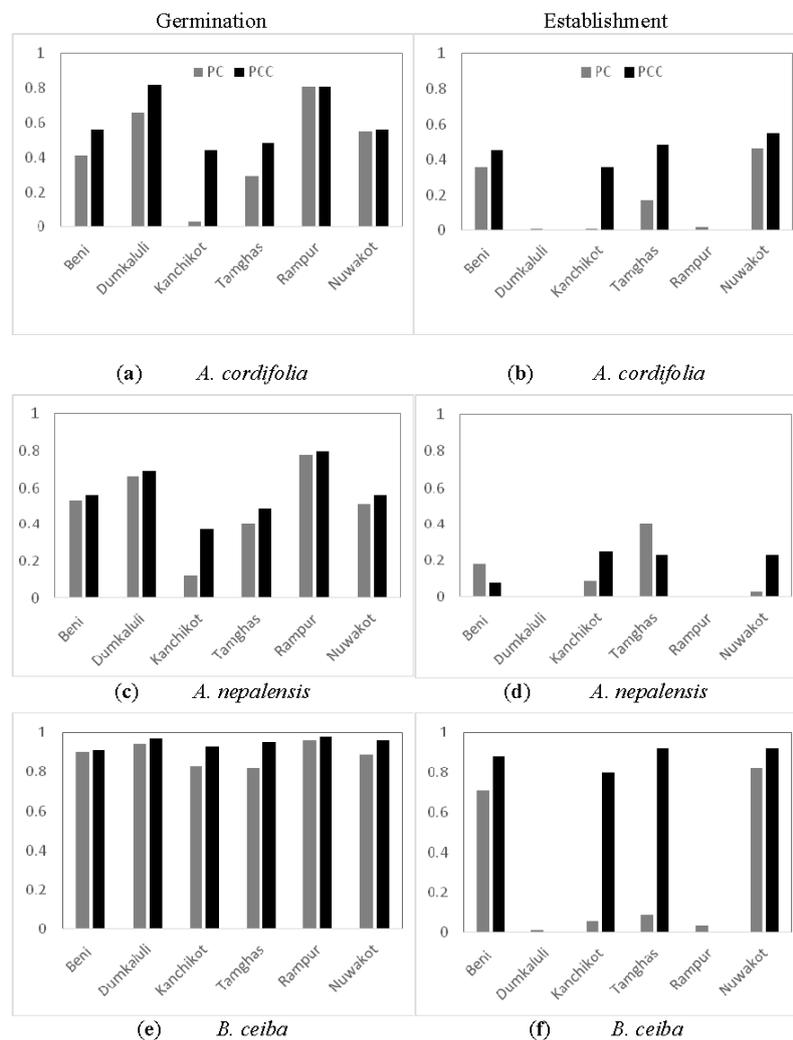


**Figure 2.** Non-linear regression prediction curve showing the growing degree days (GDDs) required for the germination of three species.

### 3.3. Species General Response:TACA-GEM

The resulting TACA-GEM model shows that the climate change by the 2060s benefitted the germination of three species across all the sites. However, the projected climatic conditions may have more of an impact on the establishment potential of most of the species (Figure 3). The lower establishment scores exhibited by all three species at Dumkauli and Rampur under the PC that resulted in zero establishment under PCC indicates the spatial vulnerability of the species at these sites.

Generally, PC and PCC benefitted establishment across warm (Nuwakot, Beni) and cool areas like Kanchikot and Tamghas for *A. cordifolia* and *B. ceiba* (Figure 3a,b,e,f). The model resultant demonstrated that for these two species there is more chance of establishment success in cool areas than in the warm areas under the PCC. However, a mixed response has been demonstrated by *A. nepalensis* as establishment declined under the projected climate change at Tamghas and Beni while it increased at Kanchikot and Nuwakot (Figure 3c,d) indicating species sensitivity to environmental cues like temperature and rainfall or other factors.



**Figure 3.** Germination and establishment probability score (0–1) indicating a scale ranging from no germination to successful germination/establishment of *A. cordifolia* (a,b), *A. nepalensis* (c,d), and *B. ceiba* (e,f), across six sites under past (PC) and projected (PCC) climate conditions.

## 4. Discussion

### 4.1. Spatial Response

Climate change is one of the key drivers changing and leading to biodiversity loss. Shifting habitat, changing the timing of life cycle events, and developing new physical traits are the major forms of species climate change, representing adaptation response [15]. Under such situations the study of physical traits linked to adaptation response and resiliency of species against climate change provides a crucial guideline for the conservation and sustainable management of forests.

For this study, implementation of a mechanistic modeling tool linking species germination phenology with the ecological niche of the habitat demonstrated how species germination and establishment trait response may change under projected climate change, helping to identify species resiliency to PCC. The resulting model showed that generally PCC across the hot tropical areas may result in greater vulnerability in species establishment than under the warm and cooler areas of these study sites. For example, low establishment potential and failure of establishment for most of the species under the PCC across hotter sites like Dumkauli and Rampur suggest that these sites may become more vulnerable to climate change in the future. Dumkauli and Rampur have higher temperature conditions than the other study sites, and high temperature conditions leading to increased evaporation can make the soil conditions dry, consequently affecting species establishment and physiological activities [57,58]. However, the study demonstrated that there is likely chance that these species may become more prevalent in forests where the climatic conditions are moderately warm or cool (Beni, Nuwakot, Tamghas, Kanchikot) under PCC.

Germination represents a high-risk transition phase and environmental conditions are the key drivers affecting germination [18,19,59]. Germination decline may lead to lower seedling recruitment and seed production, ultimately affecting plant diversity and composition [19,20]. Based on model simulation, this study demonstrates that species recruitment success relied more on spatial climatic conditions than germination, consequently indicating that species germination success may not guarantee establishment or recruitment success [36]. Hence, the study indicated that although species may exhibit germination success, the failure of establishment may result in recruitment decline, ultimately affecting species composition in the forest within Dumkauli and Rampur. Thus, the model results demonstrated that a spatial climatic pattern is crucial to limiting species regeneration and recruitment, affecting species abundance and forest composition. Therefore, the spatial climatic pattern and its impact has to be given strong consideration, and adaptation planning at a regional scale has to be strengthened [60]. Initiatives should be taken to prioritize adaptation, focusing on vulnerable regions [61].

### 4.2. Species-Specific Response

Two of the studied species, *A. cordifolia* and *B. cieba*, inhabit and are dominant across the tropical and subtropical forests of Nepal ([53,56], [www.forestrynepal.org](http://www.forestrynepal.org)). The regeneration and recruitment responses exhibited by *A. cordifolia* suggest that moderately warm climatic conditions are more favorable for this species, although the current distribution suggests that this species also inhabits the hot tropical forests of Nepal ([www.forestrynepal.org](http://www.forestrynepal.org)). Therefore, the result indicates that although the current regeneration and diversity of *A. cordifolia* may be diverse in hot subtropical and tropical forests of Nepal, under the temperature increment of PCC this species regeneration and recruitment may become risky and its diversity may expand in the forests with moderately warm and cooler climatic conditions.

The regeneration and recruitment enhancement demonstrated by *B. cieba* indicates this species is more resilient to climatic warming than other two species, and the wider germination temperature niche displayed by this species may help in species future abundance success [36]. The response exhibited by *B. cieba* suggests that this species regeneration and recruitment temperature niche is larger compared to the other two species studied. *Bombax ceiba* and *A. cordifolia* co-exist in the tropical regions of Nepal [56,62] and under the hot tropical climate of projected warming the diversity of

*B. ceiba* may increase more than the co-existing species *A. cordifolia*. The temperature range (Table 1(b)) of *B. ceiba* also suggests that it has wider a temperature tolerance than *A. cordifolia*, which also supports our finding.

There is a large current diversity of *A. nepalensis* in the alder forests of the subtropical regions of Nepal [38], and alder is widely found in this area [38]. Studies indicate that *A. nepalensis* has a wider range of site tolerance than its natural distribution would suggest ([www.factnet.winrock.org](http://www.factnet.winrock.org)). However, this study suggests that this species is very sensitive to climate and requires optimum moisture and temperature conditions for its recruitment. Hence, under the PCC, if there are constraints in optimum temperature and moisture conditions, then it is likely that recruitment may decline, ultimately restricting the diversity of *A. nepalensis*. Our finding thus suggests that this species may become more vulnerable to climate change at different sites of subtropical forests by the 2060s. This study provides species response to climate change based on the model results however, true species response may vary under the field conditions, and hence the study further recommends field-based observations and trials for future studies.

## 5. Conclusions

It is necessary to develop adaptation plans under anticipated climate change, accommodating the knowledge outcomes of scientific studies. However, the aspect of scientific study that demonstrates tree species response to climate change and its adaptive strategy has gained very little attention. This study provides general information on species regeneration and the recruitment niche that plays a critical role in species distribution under projected climate change. Our results, based on germination response and implementing statistical and mechanistic models, demonstrated how future climate change may have a greater impact on the recruitment of most of the species in hot areas of the Dumkauli and Rampur forests. Thus, this study was successful in providing some primary insights on how projected spatial climatic conditions may affect species regeneration and recruitment that may help to identify species vulnerability to climate change. Therefore, knowledge on spatial climatic patterns and their impact on species and adaptation planning at a regional scale have to be strengthened.

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