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Factors influencing the natural regeneration of arrow bamboo in giant panda habitat of the north Minshan Mountains, southwestern China

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To determine the effects of eco-factors on the regeneration of arrow bamboo in giant panda habitat, a field study was conducted in the Baozuo and Gonggangling Nature Reserves, Sichuan Province, China. A total of 183 quadrats (10 m×10 m) and 717 small quadrats (1 m×1 m) were investigated within the study site. Bamboo seedling density was used as an indicator of natural regeneration. Twelve factors were measured, which included topography and forest factors (elevation, slope aspect, slope degree, slope position, canopy cover, and shrub cover) and microhabitat factors (upper vegetation cover, herb cover, litter layer cover, moss cover, moss thickness, and dead bamboo density). A One-Way ANOVA was used to analyze the effects of topography and forest factors on seedling density, and a GLM (Generalized Linear Model) procedure was performed to examine the relationship between seedling density and microhabitat factors. The results indicated that elevation and canopy cover had highly significant effects on seedling density: bamboo seedling density was highest and showed the best regeneration at middle elevations (2800–3000 m) and under medium to medium-high canopy cover. Moss thickness, moss cover, and dead bamboo density were the most important microhabitat factors influencing the natural regeneration of bamboo: seedling density increased with increasing moss cover and moss thickness and decreased with increasing dead bamboo density. We propose that removal of dead bamboo and controlling grazing activities may accelerate the process of bamboo regeneration.

giant panda, staple diet bamboo, natural regeneration, eco-factors

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The giant panda, *Ailuropoda melanoleuca*, is a rare, endangered species endemic to China, whose diet consists virtually entirely of bamboo [1,2]. Thus a bamboo die-off after mass flowering in giant panda habitat can cause a food shortage crisis for populations of the giant panda. In the 1970s a massive flowering event of bamboo in the Minshan Mountains resulted in the death of 138 pandas [3]. The rate and quality of natural regeneration of these bamboo species following a die-off determines the length of the food shortage crisis for the pandas. Improving the rate and quality of natural regeneration of bamboo species after a mass die-off is critical for the conservation of giant pandas and their habitat [4].

Bamboo plants die-off over a large area after mass synchronous flowering. Restoration of these bamboo stands depends solely on natural regeneration from seeds [5,6]. This natural regeneration process depends on the rate of seed germination under suitable climate and soil conditions

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and on seedling survival and growth back to their original full size over several years [7,8]. The quality and rate of the natural regeneration and restoration of the bamboo forest stands are determined not only by the biological characteristics of the bamboo species but also by the environmental factors which affect seed germination and seedling growth [8–10]. Previous studies on natural regeneration of staple diet bamboo mainly focused on the biological characteristics of seeds and seedling growth [11] and the growth of young bamboo [12–14]. Unfortunately, the relationship between eco-factors and natural regeneration of bamboo has received less attention. Li [8] and Ma and Wang [15] analyzed the effect of eco-factors on seedling regeneration in the Wanglang Nature Reserve, but their results were not completely consistent with each other. Further research is needed.

In the giant panda habitat of the north Minshan Mountains, a massive flowering event of bamboo took place in 2004 and 2005 with diminished levels of flowering occurring through 2007. The area of the mass flowering of bamboo was 73082 ha. The bamboo in this area consisted of only two species of arrow bamboo, *Fargesia nitida* and *F. denudata*, which are critical staples of the pandas [16]. After such a bamboo flowering and die-off event, panda habitat in this area was no longer suitable for pandas. Following the 2004 mass flowering in the Baozuo and Gonggangling Nature Reserves, we conducted a systematic field study in the forest area of the bamboo die-off. This study had three principal goals: (1) estimate the regeneration quality of bamboo forest stands in the giant panda habitat of the north Minshan Mountains; (2) determine the effects of eco-factors on the regeneration of arrow bamboo (*Fargesia nitida* and *F. denudata*); and then (3) provide useful proposals to promote the recovery of bamboo forest stands and giant panda habitat in this area.

1 Materials and methods

1.1 Study area

The study was conducted in two connected giant panda habitats of the north Minshan Mountains, which are located in the Gonggangling Nature Reserve (103°24′–103°48′E, 33°02′–33°44′N) and the Baozuo Nature Reserve (103°12′–103°37′E, 33°03′–34°39′N) in Sichuan Province, China. The climate of the study site is warm with an annual mean temperature of 12.7°C and an annual mean precipitation from 600 to 800 mm [16]. The vegetation within the study area consists mainly of evergreen coniferous trees such as *Picea purpurea*, *P. asperata*, and *Abies faxoniana* [16].

The giant panda's staple diet bamboo species, *Fargesia nitida* and *F. denudata*, are distributed under the coniferous forest at the elevation of 2400 to 3500 m, and flowered simultaneously in 2004 [16]. Generally, the two species are often distributed in mixed species stands, which makes it difficult to distinguish seedlings by species, so in this study we lumped the seedlings of both species into one operational taxon called arrow bamboo for the purpose of field investigation.

1.2 Field data collection

Measurement was conducted in the Baozuo Nature Reserve in June–July 2010 and in the Gonggangling Nature Reserve in June–July 2011. Twenty-six transect lines (parallel or perpendicular to the orientation of valleys) were established in the forest where the arrow bamboo had flowered. Each transect line was about 1–5 km long to cover as many bamboo dominated zones as possible. We set up 10 m×10 m quadrats at intervals of at least 20 m on each transect line and all quadrats distributed at the elevation of 2500–3300 m. In each quadrat, we recorded the elevation (measured with a handheld GPS receptor), slope aspect and slope degree (measured with a compass), slope position, and canopy cover (%). Each large 10 m×10 m quadrat was further divided into 4 medium quadrats measuring 5 m×5 m to estimate the shrub cover (%). We used the average shrub cover from the medium quadrats of each large quadrat as the shrub cover of the large quadrat. Three to five small quadrats of 1 m×1 m were set up within each 10 m×10 m quadrat according to the distribution of bamboo (i.e., the presence of live or dead bamboo). Our standard protocol was to place 5 small quadrats within each large 10 m×10 m quadrat: one small quadrat was set up in the center of each medium quadrat and an additional fifth small quadrat was set up in the center of the 10 m×10 m quadrat (Figure 1). In the cases where areas within the large quadrat showed no evidence of bamboo presence, small plots that would have been placed over those areas without bamboo were not included, which is why some large quadrats contained only 3 or 4 small quadrats that were sampled instead of the full complement of 5 small quadrats. Within each small quadrat, we measured the seedling height (cm), seedling density (stems/m^2), upper vegetation cover (%), herb cover (%), litter layer cover (%), moss cover (%), moss thickness (cm), and dead bamboo density (stems/m^2). The recorded factors are listed in Table 1.

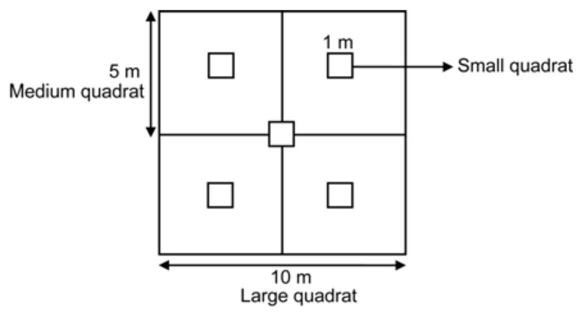

Figure 1 The design and position of medium and small quadrats within each large 10 m × 10 m quadrat.

Table 1 Factors for analysis concerning topography, forest characteristics, and microhabitat

Classification of eco-factors	Variables	Notes
Topography factors	Elevation (m)	Measured values were divided into 3 levels: <2800, 2800–3000, >3000
	Slope aspect (°)	Measured values: shady slope(337.5°–67.5°), sunny slope(157.5°–247.5°) , half-sunny and half-shady slope(67.5°–157.5°、247.5°–337.5°), no aspect (flat ground or concave ground)
	Slope degree (°)	Measured values were divided into 5 levels: <5°, 6°–20°, 21°–30°, 31°–40°, >40°
	Slope position	4 types: upper, middle, and lower slope, and valley
Forest factors	Canopy cover	Estimate values were divided into 5 levels: <0.2, 0.2–0.40, 0.41–0.6, 0.61–0.8, >0.8
	Shrub cover (%)	Dead bamboo stems were excluded. Estimate values were divided into 4 levels: 0–20%, 21%–40%, 41%–60%, >60%
Microhabitat factors	Upper vegetation cover (%)	Cover created by all vegetation above the bamboo seedlings including both trees and shrubs (dead bamboo stems were excluded). Estimate values to the nearest 10%
	Herb cover (%)	Estimate values to the nearest 10%
	Litter layer cover (%)	Estimate values to the nearest 10%
	Moss cover (%)	Estimate values to the nearest 10%
	Moss thickness (cm)	Estimate values to the nearest 0.5 cm
	Dead bamboo density (stems/m^2)	Measured values: the number of standing dead bamboo stems in small quadrat

Seed germination generally appeared early in the first 3–5 years after bamboo flowering. After 5 years the germination rate declines and the seeds lose their germinal ability [11,17]. At the same time, in our study sites, the seedling height and seedling density were positively correlated (Baozuo: r=0.286, P<0.001; Gonggangling: r=0.455, P< 0.001). Thus, we used the bamboo seedling density as a good indicator of natural regeneration. We used the average density from the small sample quadrats of each 10 m×10 m quadrat as the dependent variable when analyzing the relationships between seedling density and topography and forest factors. The relationship between seedling density and microhabitat factors was analyzed using the data from the individual small quadrats directly within each 10 m×10 m quadrat.

1.3 Statistical analysis

We used a non-parametric Mann-Whitney U Test to examine differences of seedling densities between the Gonggangling and Baozuo Nature Reserves, but no significant difference was observed in the seedling densities between the two study sites (P=0.078>0.05), we therefore pooled the data of the two sites for analysis.

We used a One-Way ANOVA to test the effects of topography variables and forest variables on seedling density. Primary data were square root transformed prior to analysis to meet assumptions of normality and homogeneity of variance. Differences were considered significant at the P < 0.05 level.

We used a Spearman rank correlation coefficient to assess colinearity between variables. Correlations between pairs of variables with magnitudes greater than ±0.5 indicate high colinearity [18]. The variables which provided the most meaningful biological interpretation were retained and others removed from further analysis [19].

To examine the relationship between seedling density and microhabitat factors, we performed a GLM (generalized linear model) procedure for this analysis, using seedling density as the response variable and microhabitat variables as the explanatory variables.

All analyses were conducted in SPSS 16.0.

2 Results

2.1 Assessment of the regeneration quality of bamboo forest stands

A total of 183 plots (10 m×10 m) and 717 small quadrats (1 m×1 m) were investigated in the study site. The seedling density ranged from 0 to 465 stems/m^2, but the majority of the seedling densities were considered low (moderate or poor), with more than three-quarters of all plots having less than 80 stems per m^2 (59.93±2.40 stems/m^2). As can be seen from Table 2, among the total of 717 small quadrats, 46.3% were evaluated as showing "poor regeneration" and 27.2% and 23.29% were considered "moderate" and "good", respectively. Quadrats which had no seedlings only account for 3.21%.

Generally speaking, in our study area, the quality of regeneration was poor in about 50% of all of the small

Table 2 Estimation of the regeneration quality of bamboo forest stands

Classification	Seedling density (stems/m^2)	Gonggangling		Baozuo		Total	
		No.	Percent (%)	No.	Percent (%)	No.	Percent (%)
No regeneration	0	10	2.82	13	3.58	23	3.21
Poor	<40	148	41.81	184	50.69	332	46.3
Moderate	40–80	109	30.79	86	23.69	195	27.2
Good	>80	87	24.58	80	22.04	167	23.29

quadrats for both the Gonggangling and Baozuo Nature Reserves, whether analyzed jointly or separately (Table 2).

2.2 The effects of topography and forest factors on the regeneration of arrow bamboo

We found that slope aspect ($F_{3,179}$=0.519, P=0.67), slope degree ($F_{4,178}$=1.83, P=0.125), slope position ($F_{3,179}$=0.363, P=0.78), and shrub cover ($F_{3,179}$=0.505, P=0.68) had no significant effects on seedling density (Table 3).

Elevation and canopy cover, however, had significant effects on seedling density ($F_{2,180}$=3.17, P=0.044<0.05 and $F_{4,178}$=4.094, P=0.003<0.01, respectively) (Table 3). The seedling density was significantly higher at middle elevations (68.97±6.13 stems/m^2) than at higher or lower elevations (47.9±6.20 stems/m^2 and 59.41±5.57 stems/m^2, respectively) (Figure 2(a)). The seedling density was highest under the medium and medium-high levels of canopy cover (canopy cover of 0.41–0.60 and 0.61–0.80) and significantly higher in these two medium to medium-high levels than under either the lowest canopy cover (<0.2; 44.3±5.37 stems/m^2)or the highest canopy cover (>0.8; 51.55±10.42 stems/m^2)(Figure 2(b)).

2.3 The effects of microhabitat factors on the regeneration of arrow bamboo

The 6 microhabitat variables were not independent from each other (Table 4): the moss cover and litter layer cover were highly negatively correlated with each other (r=–0.627). In order to deal with the colinearity due to the pairwise correlations between explanatory variables, we removed the litter layer cover so that the remaining variables are not highly correlated and, therefore, could be appropriately entered into further analysis.

We then performed a GLM procedure in which upper vegetation cover, herb cover, moss cover, moss thickness, and dead bamboo density were included in the first set of analyses (Table 5, extended models). When upper vegetation cover and herb cover generated P-values of 0.143 and 0.859, we used backward elimination procedures (eliminate predictors with P>0.10) and repeated the analyses without entering upper vegetation cover and herb cover.

As evident from the Reduced models in Table 5, the moss cover, moss thickness, and dead bamboo density

Table 3 Summary of One-Way ANOVA results with seedling density as dependent variables and topography variables and forest variables as independent variables

Variables	d*f*	*F*	$P^{a)}$
Elevation	d*f*1=2, d*f*2=180	3.17	0.044*
Slope aspect	d*f*1=3, d*f*2=179	0.519	0.67
Slope degree	d*f*1=4, d*f*2=178	1.83	0.125
Slope position	d*f*1=3, d*f*2=179	0.363	0.78
Canopy cover	d*f*1=4, d*f*2=178	4.094	0.003**
Shrub cover	d*f*1=3, d*f*2=179	0.505	0.68

a) *P<0.05; **P<0.01.

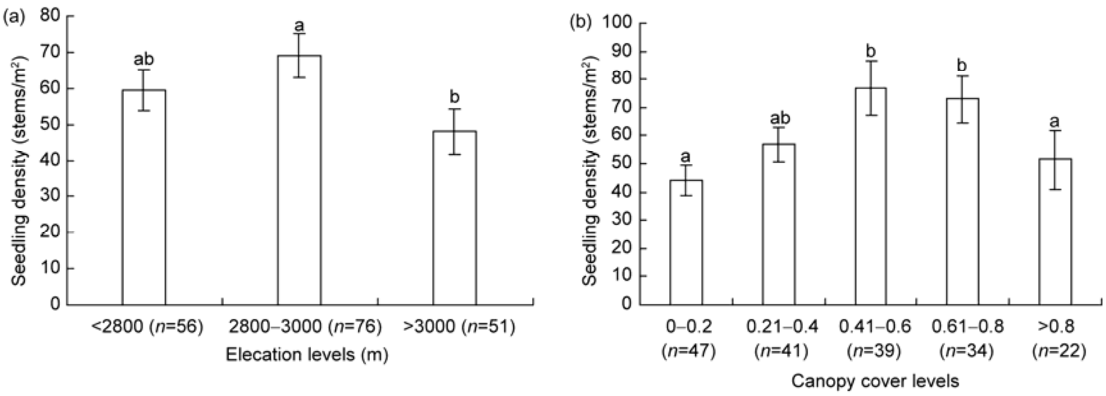

Figure 2 Mean(±SE) seedling density of arrow bamboo in different elevation and canopy cover levels. Means with different letters indicate significant differences at the P < 0.05 level.

Table 4 Spearman correlation coefficient between microhabitat factors[a)]

	Variables	X_1	X_2	X_3	X_4	X_5	X_6
X_1	Upper vegetation cover (%)	1	–0.043	0.259**	0.037	–0.026	–0.007
X_2	Herb cover (%)		1	–0.121	0.081*	–0.053	–0.040
X_3	Litter layer cover (%)			1	–0.627**	–0.455**	0.021
X_4	Moss cover (%)				1	0.332**	–0.039
X_5	Moss thickness (cm)					1	–0.064
X_6	Dead bamboo density (stems/m^2)						1

a) *P<0.05; **P<0.01.

Table 5 Analysis of microhabitat variables affecting natural regeneration of arrow bamboo. The response variable in all models is the number of seedlings per m^2

Variables	B	SE	Wald $\chi 2$	d*f*	*P*
Extended models					
upper vegetation cover	0.000	0.0001	2.141	1	0.143
herb cover	–0.028	0.1556	0.032	1	0.859
moss cover	0.277	0.1234	5.027	1	0.025
moss thickness	0.168	0.0242	48.233	1	<0.001
dead bamboo density	–0.003	0.0011	7.729	1	0.005
Reduced models					
moss cover	0.298	0.1137	6.893	1	0.009
moss thickness	0.176	0.0220	64.259	1	<0.001
dead bamboo density	–0.003	0.0010	9.070	1	0.003

remained the factors with the strongest influence on the seedling density. The seedling density increased with increasing moss cover and moss thickness; whereas seedling density decreased with increasing dead bamboo density.

3 Discussion

Our results showed that elevation and canopy cover had highly significant effects on seedling density. The seedling density at elevations from 2800 to 3000 m was higher than the density below 2800 or above 3000 m, which implies that elevations from 2800 to 3000 m may be optimal where the moisture, temperature, and light conditions are most favorable for bamboo regeneration.

Seedling density was highest under medium (0.41–0.6) to medium-high (0.61–0.8) canopy cover and the seedling densities were higher in these two levels than under either the lowest canopy cover (<0.2) or the highest canopy cover (>0.8). Previous studies of *F. denudata* in Wanglang Nature Reserve reported that the bamboo seedlings showed the best regeneration under a medium canopy cover [8,17]. Our result was similar to theirs. Canopy cover was an important factor which was expected to influence forest understory conditions such as light, temperature, and moisture [20]. When living in a more shady environment (under higher canopy cover), it was difficult for seedlings to get enough light, which would likely result in lower growth rates and lower survival [15]. However, under more open canopy conditions, the seedlings face competition from weeds and shrubs that benefit from sunny conditions and grow better in such habitat and which likely compete with bamboo seedlings for nutrients and water [17,21].

Our results also showed that moss thickness, moss cover, and dead bamboo density were the most important microhabitat factors influencing the natural regeneration of bamboo (Table 5, reduced models).

Moss thickness and moss cover had significant influence on the seedling density and the seedling density increased with increasing moss thickness and moss cover. Our result was consistent with results of Li [8], who also reported that the vigorous growth of bamboo seedlings would be suppressed by moss thickness greater than 15 cm. In our study site, the moss thickness we measured varied from 0 to only 10 cm, and, therefore, the seedling density showed an increase with increased moss thickness. Ma and Wang [15] reported that the bamboo seedlings showed the best regeneration when living in the environment with extremely thin moss layer and the seedling density decreased with increasing moss thickness. Our and Li's results were both contrary to Ma's findings, perhaps because Ma's study was based on a small sample of only 17 standard plots, which cannot fully reflect the overall situation. Some uncertainties may remain in his conclusion. As the main ground cover of many ecosystems, bryophytes play an important role in the process of vascular plants' natural regeneration. The microclimate changes (temperature, air moisture, soil moisture, etc.) induced by the physiological characteristics of bryophytes could affect seed germination and seedling growth [22]. While the lack of a moss layer or an overly thick moss layer may not beneficial to the early stages of plant regeneration. The existence of a moss layer with a moderate thickness may have had a beneficial effect on bamboo regeneration because the fallen mature seeds are mainly stored in the moss layer. The advantages of the moss layer for maintaining humidity and being non-pathogenic may have improved the rate of bamboo seed germination, thus enhancing the possibility of bamboo forest generation [8]. However, living in thicker moss layers, such as greater than 15 cm, may impede the ability for seedling roots to reach the humus layer, and thus possible lack of water, nutrients, and light might reduce seedling growth and survival [22].

In our study, dead bamboo had a negative influence on seedling regeneration. A previous study of *Bashania faberi* in the Wolong Nature Reserve found that regeneration and growth of young bamboo were restrained in zones dominated by dead bamboo [12]. After bamboo flowers and dies, their underground root disappears over time; however, this decomposition process is slow. The presence of dead bamboo inhibited seedling growth by decreasing available space both underground and above ground. Furthermore, dense dead bamboo might affect the light conditions for germinating bamboo seeds and seedlings. Thus dead bamboo remains likely restrained or blocked the regeneration process.

In our study, we found that microhabitat factors such as moss thickness, moss cover, and dead bamboo density were the most important factors influencing the regeneration of bamboo. After bamboo flowers and dies, restoration of bamboo forest stands depends solely on seeds. During the period of seedling establishment after germination, many seedlings failed to form a sufficiently robust root system and thus their respiration and absorption of nutrients were limited. This very small-scale microhabitat has its own specific light, temperature, moisture, micro-topography, and competition conditions [23]. Consequently, microhabitat has a primary effect on seedling establishment and survival. Our results also found that dead bamboo had a strong negative impact on seedling density at the microhabitat scale, indicating that removal of dead bamboo may accelerate the process of bamboo regeneration.

In addition, we found that grazing activity is intensive in the areas of our study. Such grazing activity may easily result in destruction of the moss layer and the death of bamboo seedlings from animal trampling. Measures should be taken to control grazing activities in forest areas in the nature reserves in order to promote and enhance the regeneration of bamboo forest stands.

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