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Seed yield predictions based on the habitat niche-fitness of *Microula sikkimensis*, an endemic oil crop in the Qinghai-Tibet Plateau

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ABSTRACT. *Microula sikkimensis* is a biennial herb, found only in the eastern rims of the Qinghai-Tibetan Plateau, and lends itself to multiple applications in medicine, food and fodder. Utilizing the seed production of *M. sikkimensis* to develop a sustainable production ecosystem is a logical option for the degraded alpine grassland in the Qinghai-Tibet Plateau. From 1994 to 2004, a field survey and transplanting trials were conducted in eleven counties of three provinces for collection of habitat factor data. By multivariate statistical analysis the habitat factors were condensed into seven key habitat factors which described an actual habitat state. Introducing the niche theory into the research of *M. sikkimensis*, habitat niche-fitness (HNF) is defined as the degree of similarity of an actual habitat state to the optimum habitat. A new model of HNF is constructed to evaluate the adaptive extent of *M. sikkimensis* and the influence of habitat on seed yield with the key habitat factors as dependent variables and factor weights as parameters. The results showed that the values of HNF had a reasonable distribution and better reflected the varied differences under different habitat conditions, and that the cultivation measures had the effect of increasing the value of HNF and seed yield, increasing 14.26% and 99.61% at an average level, respectively. A seed yield prediction model was constructed with HNF as a surrogate for composite environmental factors. The estimated seed yield agreed well with the observed data, and the average of the absolute deviation percent was 5.46%, demonstrating the validity of the model in predicting seed yield. The HNF model and seed yield prediction model evaluated the threshold value of HNF, predicted the upper limit of seed yield for each study site and the limit seed yield, and have a wide range of prospects for practical application in the similar regions of the Qinghai-Tibet Plateau.

Keywords: Habitat niche-fitness (HNF); *Microula sikkimensis*; Seed yield; The Qinghai-Tibet Plateau.

INTRODUCTION

The Qinghai-Tibet Plateau, located in southwest China, has a unique, high-altitude natural environment and is richly endowed with natural resources. Most of the area is alpine steppe and meadow (1,627 million km^2). These ecosystems play an important role in agriculture, water conservation, biodiversity, and ecological safety. However, they are also extremely fragile on account of steep inclines, sparse vegetation, severe physical air-slaking conditions, strong solar radiation, loose soil texture, low fertility, and eroded soil. In the past decades, they have suffered many eco-environmental problems caused by long-term overgrazing, irrational human activities, and natural factors (climate change leading to a decrease in rainfall). At present, 61.3% of this alpine steppe and meadow has degenerated and is progressively converting to barren land. This has dramatically reduced agricultural production and resulted in poverty for the human inhabitants. Ways to develop these ecosystems commercially and environmentally are urgently needed. Any solution will need to take into consideration both the need to increase agricultural production and farmer income and the requirement for long-term sustainability of the farming system. Exploiting the plentiful availability of plant germplasm may be a key factor in creating a breakthrough in achieving these purposes (Ren and Lin, 2005).

The Qinghai-Tibet Plateau is home to 25 wild species of the endemic genus *Microula* Benth. in the family *Boraginaceae*. The other four species can only be found in the alpine regions of Sikkim, Nepal, Bhutan, and Kashmir (Wang et al., 1997a). *Microula sikkimensis* is one of the 25, a biennial herbage rich in γ-linoenic acid. As a stenotopic species, it was mainly confined to the eastern rims of the Qinghai-Tibetan Plateau (including Gansu, Qinghai, and Sichuan Provinces) and was typically associated with degraded alpine steppe and meadow, especially at the beginning of the secondary succession (Wang et al., 2003b).

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It possesses multiple applications as medicine, food, and fodder. Pharmaceutical experiments have indicated that its seed oil can significantly decrease cholesterol and trilycerides in blood serum, increase the ratio between total cholesterol and high-density lipoprotein, prevent the accumulation of atheroma, preserve the structural integrity of biological membranes and inner membranes of vessels, and alleviate high blood fat (Li et al., 1999a, b). The coarse stalks of *M. sikkimensis* contain abundant mineral nutrient elements and crude protein (Wang et al., 2003b) and can be processed into fodder. It has been estimated that high profits could be made using this new feed resource to raise animals in winter and spring (Zhang and Deng, 1999b). Therefore, exploiting this new promising oil crop to develop a sustainable production ecosystem is both a logical and natural option for the degraded alpine steppe and meadow ecosystems in the Qinghai-Tibet Plateau. However, this plant is not yet utilized as a crop because of its low seed output in nature; in fact, it is even eliminated as a weed. Consequently, this species has faced the threat of extinction for several decades. The initial questions related to utilization and development of *M. sikkimensis* for farmers are: whether the location of interest is a suitable habitat for cultivation of this species or not, whether an acceptable seed yield can be harvested, and what the potential seed yield will be when agricultural cultivation technology is applied. Therefore, research on habitat factors that determine seed yield and appropriate habitats for cultivation of this species is required. Such research could improve seed yield, encourage a potentially new, ecologically sustainable industry, and predict where the promising new crop will produce well.

A list of a species' habitat requirements can be used to predict the species' presence or absence. From the perspective of plant production, the factors that affect plant growth fall into two categories: habitat factors—of which temperature, water, and soil conditions are most important—and agricultural cultivation technology. Under the same cultivation conditions, it is obvious that habitat factors play a key role in the existence and productivity of a plant. These or associated habitat factors could be used to identify suitable and unsuitable habitats for planting (Li and Lin, 1997; Lin and Li, 1998; Buggeman and O'Nuallain, 2000; Liu et al., 2000). In general, the interactions between habitat factors that affect the productivity of plants are complex, multi-factorial, and often difficult to describe mathematically, posing a challenge for seed yield predictions based on traditional methods (Li and Lin, 1997; Lin and Li, 1998). The fact that the seed yield regressed directly with habitat factors under regression-type models has lead them to be criticized for their empirical nature (Li and Ren, 1997; Scian and Bouza, 2005), which created some difficulties in determining whether *M. sikkimensis* could be introduced or not. It is difficult to base an such an appraisal on direct judgments alone (Jiang and Wang, 2004). Up to now, a number of scholars have explored the physiological and ecological characteristics of *M. sikkimensis* in such aspects as its distribution and population characteristics (Wang et al., 1997a; 1998a, b; 2003b), physiological traits (Niu, 1997; Wang et al., 1997b, 1998c, d, e; Sun and Wang, 1998), chemical composition in seed (An and Zheng, 1996; Fu et al., 1997, 1999; Zhen et al., 1997), medical value (Li et al., 1999a, b), nutritional value (Zhang and Deng, 1999b), and breeding of cultivars (Zhang and Deng, 1998, 1999a; Wang et al., 2003a). However, quantitative research on distribution regularities and habitat factors that determine seed yield has not been done.

Understanding the ecological adaptability of the species to different habitats is helpful for successful conservation and is also useful for breeding for direct utilization. Our work had three main goals: the first was to extract the key habitat factors i.e., factors having significant influence on the seed yield of *M. sikkimensis* by Pearson Correlation and Partial Correlation Analyses. The second goal was to discuss the meaning of habitat niche-fitness (HNF) and construct its mathematical model for this plant with the key habitat factors as dependent variables and factor weights as parameters. The last was to establish a prediction model for seed yield and to validate the prediction model. It is hoped the HNF model and seed yield prediction model will provide farmers and practitioners with a viable tool for identifying likely areas for cultivation of this species and applying the right methods of fertilization, utilization, and development of *M. sikkimensis* in similar regions of the Qinghai-Tibet Plateau.

MATERIALS AND METHODS

From April 1994 to September 2004 field surveys were carried out at eleven sites over a wide area of the eastern rims of the Qinghai-Tibetan Plateau, using the species' responses to habitat factors as reliable criteria in an analysis. In this study, transplanting trials adopting a recommended cultivation technology resulted in habitat modification. By transplanting trials at five sites selected from the eleven field survey sites, the effects of habitat modification were examined. Durations of field surveys and transplanting trials (Month/Year) are shown in Table 1. Basic descriptive statistics of range, mean, and standard error on the habitat factors are shown in Table 2.

Field surveys

The field survey sites were chosen in abandoned fields of degenerated alpine steppe and meadow where *M. sikkimensis* had reached dominance in secondary succession. The survey sites were situated in Hongyuan, Ruoergai, Aba, and Maerkang Counties in Sichuan Province, Tianzhu, Hezuo, and Maqu Counties in Gansu Province, and Menyuan, Haiyan, Gangcha, and Huangzhong Counties in Qinghai Province (geographical coordinates from 100º08' to 104º27'E and 31º50' to 37º22'N). These sites were selected because (1) they were the most representative sites in degenerated alpine steppe and meadow; (2) they were

Table 1. Meteorological and geographic features of the study sites.

Site	North latitude (ºN)	Altitude (m)	Maximum mean temperature of Jul. (°C)	Minimum mean temperature of Jan. (°C)	Annual>0°C accumulated temperature (°C)	Annual mean precipitation (mm)	Annual mean temperature (°C)	Duration of field survey (Month/ Year)	Duration of transplanting trial (Month/ Year)
Huangzhong	36º25'	2667.5	12	-8	2065	527.6	2.8	4-9/2000	
Maqu	34º24'	2707.6	11.9	-15.4	1422.6	514.5	0.5	4-9/1997	4-9/2002
Hezuo	35º05'	2915.7	12.6	-9.8	1729.3	558.1	0	4-9/1997	
Tianzhu	37º18'	3045.1	11.3	-11.4	1327.7	411.3	-0.2	4-9/1996	4-9/2001
Haiyan	36º56'	3080	7.5	-18.1	1528.6	397.44	-0.3	4-9/1999	4-9/2004
Aba	32º54'	3275	12.15	-5.85	1891.9	712	3.2	4-9/1995	
Gangcha	37º20'	3301.5	10.6	-13.6	1225.2	377.1	-0.6	4-9/2000	
Ruoergai	33º20'	3446	9.6	-9	1518.3	651.3	0.6	4-9/1994	
Mengyuan	37º27'	3471.6	10.7	-9.2	1404.4	615.5	1.4	4-9/1998	4-9/2003
Hongyuan	32º48'	3504	10.1	-8.38	1432.2	728.4	1.1	4-9/1994	4-9/1997
Maerkang	31º50'	3664.4	16.2	-0.4	3192.2	753.1	8.6	4-9/1995	

Note: Meteorological data for each study site derived from located grassland research stations were calculated for a ten-year period (1994-2004).

within the major *M. sikkimensis* distribution areas; and (3) their habitat characteristics varied greatly. Their meteorological and geographic features are shown in Table 1. Their weather typifies the continental plateau climate. The growth season is very short, and total no-frost time is 70 to 190 days annually. Soil types are alpine steppe and meadow soils. Consequently, it is difficult for trees to survive in this environment, which belongs to the alpine herbosa zone and is dominated by psychrotolerant vegetation.

The vegetative, flowering, and withering stages of *M. sikkimensis* are late April to early May, late June to early July, and mid to late September, respectively. In each field survey site, five subplots of 1 m × 1 m were randomly selected with a distance of 50 m between subplots. Soil and vegetative attributes were observed once within subplots in different growing season stages. The method of timing and positioning, commonly accepted in agricultural science, was used in observing the fields for field study of *M. sikkimensis* growth and seed yield indexes. In mid September seeds of *M. sikkimensis* were harvested, dried, and weighed to the nearest 0.01 g. Soil samples were taken at the 0-30 cm layer in the subplots for different growing stages. Soil analysis and extraction were carried out by commercial laboratories adopting standard measurement techniques (Honda, 1962; John, 1970; Black, 1979; Olsen and Sommers, 1982; Gee and Bauder, 1986). Soil factors measured were texture, soil moisture (SM), organic matter (OM), soil acidity (pH), soil salinity (electrical conductivity, EC), total nitrogen (TN), alkaline hydrolytic nitrogen (AHN), total phosphorus (TP), available phosphorus (AVP), total potassium (TK), and available potassium (AVK).

Transplanting trials

The transplanting trials were performed in locations corresponding to the field survey sites in Hongyuan County of Sichuan Province, Maqu and Tianzhu Counties of Gansu Province, and Menyuan and Haiyan Counties of Qinghai Province. Each transplanting plot at the different sites was 10 m × 10 m with 1 m buffer strips with four replicates. Root tubers of *M. sikkimensis* supplied by Gansu Grassland Ecological Research Institute via quick regeneration multiplication tissue culture (Wang et al., 2003a) were transplanted by hand in mid-April. Considering secure fertilizer criterion (Lu and Yang, 1997), sheep manure at a rate of 7,500 kg/ha were applied before transplanting. Fertilizer nitrogen (urea, 46% N) at a rate of 86 kg/ha and fertilizer phosphorus (super phosphate, 12% P_2O_5) at a rate of 54 kg/ha were split into three equal amounts. The first amount was added during the land preparation prior to transplanting; the second was added 30 days after transplanting, and the final amount at cyme initiation. Because the soil of the study region is rich in potassium, none was applied. Weeding was performed

Table 2. Descriptive Statistics of habitat variables.

Habitat variables	Abbr.	Unit	Range	Mean value	Standard error	C.V. (%)*
North latitude	NL	ºN	31 º35'-37 º27'	35º01'	2º17'	6.22
Altitude	AL	m	2667.5-3664.4	3188.95	331.99	10.41
Maxium temperature of Jul.	MT	°C	17.5-26.2	11.33	2.17	19.15
Minimum temperature of Jan.	NT	°C	-38.1 – -20.4	-9.92	4.77	48.08
Annual mean temperature	AMT	°C	-0.6-8.6	1.55	2.64	179
Annual >0°C accumulated temperature	AT	°C	1225.2-3192.2	1703.4	553.17	32.47
Annual average precipitation	AP	mm	377-753.1	567.84	135.87	23.93
Soil moisture	SM	%	11.22-68.79	23.61	9.93	32.66
Soil clay grain content (<0.01 mm)	PS	g/kg	107-159	129.5	22.5	17.37
Soil acidity	pH		4.8-8.5	6.8	2.34	34.4
Soil salinity (electrical conductivity)	EC	dS/m	2-8	6	1.8	30
Soil total nitrogen	TN	%	0.16-0.70	0.27	0.13	48.15
Soil alkaline hydrolytic nitrogen	AHN	mg/kg	21.20-50.19	30.72	10.96	35.68
Soil total phosphorus	TP	%	0.045-0.155	0.08	0.03	37.5
Soil available phosphorus	AVP	mg/kg	0.2-7.5	6.2	3.70	59.68
Soil organic matter	OM	%	6.05-22.5	12	7.75	64.58
Soil total potassium	TK	%	1.41-4.05	2.5	0.825	33
Soil available potassium	AVK	mg/kg	133-315	198.5	62.75	31.61
Plant density	PD	plants/m^2	6.67-51.20	20.19	12.31	60.97
Seed yield	Y	kg/ha	50-710	227.94	153.51	67.35

Note: *Coefficient of variation: 0-15% (least variation), 15-35% (moderate), >35% (most varied).

thrice annually. The experimental field was not irrigated. Other field management practices were identical to those for other crops grown at this area. Cultivar density was 24 plants/m^2 in Hongyuan, 16.7 in Tianzhu, 23 in Maqu, 25 in Menyuan, and 20 in Haiyan, according to soil test recommendations.

The items measured in each plot of the transplanting trials were the same as those in the field survey sites.

Data analysis methods

In order to identify habitat factors determining seed yield, Pearson Correlation among various habitat factors and Partial Correlation Coefficients between seed yield and habitat factors were analyzed with SPSS (Statistics Package for Social Science; SPSS, 1997). The principle used was that the factors that played an important role in the growth of *M. sikkimensis* would be kept as target characters (key habitat factors) while those that played the least important role would be discarded. The Partial Correlation Analysis was used for measuring the correlation between seed yield and each habitat factor while eliminating the effects of all other habitat factors in the dataset. Thus the distribution regularities could be denoted by fewer key habitat factors.

The quantitative indexes of these key habitat factors can be marked as $x_1, x_2,\ldots x_n$. The observation values of each group in natural (field surveys) or transplanting trial condition can be noted as $X = (x_1, x_2,\ldots x_n)$. X stands for a realized habitat state or a modified habitat state. Biologically, a crop will show certain adaptation to variables of each key habitat factor, so the optimum value of key habitat factor i can be marked as x_{ai} (i = 1, 2, . . ., n). x_{ai} can be obtained from experimental observation (Li and Lin, 1997; Lin and Li, 1998). $X_a = (x_{1a}, x_{2a},\ldots x_{na})$ is a quantitative description of species attributes for the optimum habitat requirements. The balance between requirement for the optimum habitat and supply of a realized habitat in the development of the species is an important characteristic and can be measured by habitat niche-fitness (HNF). Taking *M. sikkimensis* as the study object and taking the key habitat factors into consideration, we suggest that HNF for *M. sikkimensis* be defined as the degree of similarity between supply of an actual habitat and requirement for the optimum habitat, in which supply of the actual habitat and requirement for the optimum habitat denote realistic habitat conditions and species attributes, respectively. This is a measurement of the "*n*-dimensional hypervolume" defined by Hutchinson (1957). The mathematical model for HNF can be expressed as follows:

$$F = f(X, X_a, K) \quad (1)$$

In this formula, the value of HNF F, which is in the range of [0, 1], means the fitness degree of *M. sikkimensis* in an actual habitat condition. The larger the value of F, the higher the adaptive property for its habitat. Normally, when the actual habitat state may change on the large-scale eco-geographical regions, F value needs a wide distribution on the subset [0, 1]. $f(X, X_a, K)$ is the measurement of the distance or the degree of similarity between two vectors: $X = (x_1, x_2, \ldots x_n)$ and $X_a = (x_{1a}, x_{2a}, \ldots x_{na})$. However, in these rain-fed farmland systems, the importance of various key habitat factors to *M. sikkimensis* varies. Thus, we have to consider the actual conditions of unequal weights among the key habitat factors and extend Eq. (1), where vector $K = (k_1, k_2, \ldots k_n)$ is a set of weights of the extracted key habitat factors and k_i the weight coefficient of the key habitat factor i. In this study, the weight coefficient of key habitat factor is integrated by factor loadings derived from the PRINCOMP procedure of SAS (SAS, 2000). The factor loading is the correlation coefficient between the principal component and the key habitat factor, and its size depicts the key habitat factor's influence.

The data sets collected from the study sites were used to construct a seed yield prediction model by regression analyses of ln-transformed dependent (seed yield per hectare) and independent (HNF) variables. Ln transformations functioned by converting values to a scale where the variance in the relationship was more homogeneous for effective use of least-squares regression (Steel and Torrie, 1980). The seed yield prediction model with HNF as a surrogate for composite environmental factors was validated based on the statistical and biological requirements. Statistical validation was done first through the coefficient of determination (R^2), the adjusted R^2 and the standard error of the estimate. For biological verification, the observed seed yield per hectare was collectively compared with the corresponding value estimated by the seed yield prediction model with the help of absolute deviation percent. The absolute deviation percent was given by:

$$e_{ADV} = {|Y_{est} - Y_{obs}|}/{Y_{obs}} \times 100 \quad (2)$$

Where: e_{ADV} = absolute deviation percent; Y_{est} = estimated seed yield per hectare by the seed yield prediction model; Y_{obs} = observed seed yield per hectare.

RESULTS

Key habit factors selection

The seed yield was significantly correlated with the altitude (AL) and with the north latitude (NL). There was also a negative correlation between AL and NL (Table 3). In general, these survey regions with a high north latitude also had a low altitude. Partial correlation coefficients between seed yield and NL and AL suggested that AL, which was one of the indirect causes of the variability in seed yield, could be chosen as a key habitat factor, and named x_1, whereas NL is neglected (Table 4). *Microula sikkimensis* is mainly concentrated in degraded alpine steppe and meadow, so the distribution of *M. sikkimensis* is associated with altitude. It is distributed only in the altitude range from 2,600 to 5,000 m. It is discovered to have increased within areas of altitude 2,600-3,500 m, decreased in areas of altitude 3,500-5,000 m, and disappeared where the altitude is over 5,000 m. The optimum AL value x_{a1} = 3,500 m (Wang et al., 2003b).

As the growth period of *M. sikkimensis* is short (from April to September) the quantity of heat needed is an important factor. According to physiological experiment results, *M. sikkimensis* is a medium cold resistant plant that can grow at no less than 0ºC (Wang et al., 1997b, 1998c,d). The heat requirement is expressed in terms of growing-degree-days, i.e. annual ≥0ºC accumulated temperature (AT), including in the case of *M. sikkimensis*, for which the annual mean temperature (from April to September) (AMT, in ºC) is used. The cold and hot tolerances of the species are expressed in terms of minimum and maximum temperature of the coldest and warmest month (NT and MT), respectively. AMT, AT, MT and NT were significantly correlated with each other as well as with seed yield (Table 3). Absolute value of partial correlation coefficient between seed yield and AT was the biggest, so AT was chosen as a key habitat factor and named x_2 while the others were omitted (Table 4). The optimum AT value is x_{a2} = 1422ºC (Wang et al., 1997b, 1998c,d, 2003b).

Annual average precipitation (AP) and soil moisture (SM), during the *M. sikkimensis* growth cycle, had a significant correlation with each other as well as being strongly related to seed yield (Table 3), but a partial correlation coefficient between seed yield and AP was larger than that between seed yield and SM. Therefore, AP was chosen as a key habitat factor and named x_3 (Table 4). The field survey data shows that precipitation is beneficial for branching and tillering when AP reaches 400 mm. When AP is greater than 600 mm, the tillering and squaring abil-

Table 3. Correlation matrix of seed yield per hectare (Y) and habitat factors.

Habitat factors	Y	NL	AL	MT	NT	AT	AMT	AP	pH	EC	OM	TN	AHN	TP	AVP	TK	AVK	PS	PD	SM
Y	1	*	**	*	**	**	**	**	**		**	*	**	*	**				*	**
NL	-0.65	1	**		**		*	**			*	*	**	*	**					
AL	-0.73	-0.74	1																	
MT	0.52	-0.38	0.10	1	**	**	**													
NT	-0.71	-0.81	0.51	0.76	1	**	**	**			**									
AT	0.74	-0.56	0.25	0.78	0.76	1	**					**	**	**	**				**	
AMT	0.65	-0.67	0.38	0.88	0.92	0.84	1	*				*	**	*	**				*	
AP	0.81	-0.94	0.56	0.49	0.82	0.54	0.73	1				*	*		*		*		**	**
pH	-0.88	0.02	0.32	-0.02	0.32	-0.33	-0.23	-0.02	1			*		*			*			
EC	-0.37	0.00	0.01	0.15	0.33	0.15	0.37	0.44	0.38	1		*	*	*			**			
OM	0.74	0.74	0.03	-0.28	0.78	-0.14	-0.37	-0.01	-0.45	-0.06	1	**	*	**						
TN	0.63	0.56	0.05	-0.20	0.36	-0.85	-0.70	-0.43	-0.83	-0.56	0.88	1	**							
AHN	0.87	-0.75	-0.28	0.24	-0.23	0.72	0.65	0.57	-0.03	-0.53	0.62	0.85	1		**			*	**	
TP	0.70	0.59	0.39	-0.18	0.36	-0.84	-0.65	0.02	-0.65	0.54	0.88	0.24	0.24	1	**					
AVP	0.88	-0.76	-0.34	0.21	-0.21	0.73	0.79	0.61	0.02	-0.57	0.46	0.59	0.71	0.85	1				**	
TK	0.03	0.02	0.03	-0.03	0.04	-0.34	-0.23	0.03	-0.36	0.20	-0.47	0.26	0.58	0.38	0.46	1				
AVK	0.14	-0.03	-0.01	0.18	-0.04	0.14	0.20	0.66	-0.52	0.72	-0.04	-0.07	0.66	-0.05	0.20	-0.19	1			
PS	0.03	-0.48	-0.09	0.02	0.02	0.05	0.05	0.01	0.04	0.27	0.32	0.64	0.36	-0.04	0.18	-0.09	0.03	1		
PD	-0.55	-0.33	-0.48	0.27	-0.21	0.77	0.66	0.76	0.25	-0.18	0.21	0.17	0.77	0.10	0.72	0.01	0.37	0.08	1	
SM	0.77	0.47	0.38	0.02	0.34	-0.05	0.38	0.99	0.37	0.36	0.24	0.27	0.47	0.33	0.28	0.25	0.08	0.24	0.32	1

**Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed). Abbreviations are explained in Table 2 and the main text.

Table 4. Partial correlation coefficients of seed yield per hectare (Y) by habitat factors.

Habitat factor	NL	AL	MT	NT	AMT	AT	AP	pH	EC	OM	AHN	TN	AVP	TP	TK	AVK	PS	SM	PD
Partial Correlation Coefficient	-0.65	0.79	0.29	-0.14	0.54	0.83	0.77	0.73	0.39	0.60	0.70	0.65	0.69	0.66	0.03	0.22	0.32	0.59	0.67
	**	****	-	-	**	****	****	***	*	**	***	**	***	**	-	-	*	**	***

Note: Partial correlation coefficients are controlled for each of the other variables identified. $^{*}p \leq 0.10$; $^{**} p \leq 0.05$; $^{***}p \leq 0.01$; $^{****} p \leq 0.001$.

ity of *M. sikkimensis* is limited; when AP is above 800 mm, *M. sikkimensis* becomes waterlogged, and its growth is restricted, and it may even die. AP is the habitat factor affecting the moisture utilization and competition ability of *M. sikkimensis.* The optimum AP value is x_{a3} = 400 mm (Sun and Wang, 1998; Wang et al., 1998d,e).

Soil acidity (pH) was negatively correlated with seed yield (Table 3). According to results of the field survey, when pH is within 4.8-7, the distribution of *M. sikkimensis* is greatest. From 7-8.5, the distribution decreases gradually. In acid or alkaline soil with pH below 4.8 or above 8.5, there is no distribution. It has been reported that *M. sikkimensis* is very sensitive to the presence of dissolved salts in its root zone. Soil salinity (EC) in each site was far below the limit of salinity (Niu, 1997), an electrical conductivity of 16 dS/m (Table 2). Partial correlation coefficients between seed yield and pH were higher than that of EC (Table 4). Hence, pH was chosen as a key habitat factor and named x_4 while EC was omitted. According to physiochemical experiment results, *M. sikkimensis* is suitable for the somewhat acidic soils; the optimum pH value is x_{a4} = 6.5 (Niu, 1997; Wang et al., 1998d).

In Alpine steppe and meadow soil most OM derived from dead roots can reside in soils for decades due to lower temperature. TK is abundant (>15 g/kg) in the surface horizon because of a lower weathering intensity of the parent material. AVK is higher than 100 mg/kg and belongs to the top supplying potassium level. Soil clay grain content (PS) is much lower and homogeneous. The correlation and partial correlation coefficients between seed yield and TK, AVK, OM, and PS were too low to be selected as key habitat factors (Tables 3, 4). Significant correlations were observed between TN and AHN and between TP and AVP. Furthermore, seed yield and TN, AHN, TP and AVP showed significant correlations (Table 3). The partial correlation coefficients between seed yield and AHN, TN, AVP, TP are 0.70, 0.65, 0.69, and 0.66 respectively (Table 4). AHN and AVP determined the supply of soil nutrients and were chosen as key habitat factors and named x_5 and x_6, respectively, while TN and TP were neglected. Based on substrate cultivation experiments and pot experiments (Zhang and Deng, 1998; An and Ma, 2002), the optimum AHN and AVP values are x_{5a} = 60 mg/kg and x_{6a} = 24.6 mg/kg, respectively.

Plant density (PD) could be considered as a key habitat factor and was named x_7 according to its correlation and partial correlation coefficients to seed yield. The most suitable PD is x_{7a} = 25 plants/m^2 (Zhang and Deng, 1998, 1999a; An and Ma, 2002; Wang et al., 2003a, b).

Overall, the statistical analyses showed the importance of seven habitat factors that had been selected as key habitat factors from the original 19 factors, including the following controllable factors, namely AHN, AVP, pH, PD, which could be improved through agricultural management, and uncontrollable factors, namely AL, AT and AP, which could not.

Constructing habitat niche-fitness model for *M. sikkimensis*

Microula sikkimensis has the characteristic of three-base points with respect to its response to each key habitat factor, including the upper limit, the optimum value, and the lower limit, which was in accordance with the results of the field survey and transplanting trials. The nearer it approaches the region's border, the lower the fitness will be. So the value of HNF responded to variation in each key habitat factor, presenting a bell-shaped curve along its gradients. Firstly, calculating the relative degree of similarity between an actual habitat state and optimum habitat requirement, the HNF model can be constructed, which is intuitively and mathematically meaningful, as follows:

$$F = \sqrt{\sum_{i=1}^{7} k_i \min\{(\frac{x_i}{x_{ai}})^2,\ (\frac{x_{ai}}{x_i})^2\}} \quad i = 1 \cdots 7 \quad \text{...(3)}$$

In Eq. (3), the value of HNF, *F* represents the degree of similarity of an actual habitat to the optimum habitat, which reflects the demand-supply relation between plant growth and its habitat resources. x_i is the actual state of key habitat factor *i*. In order to emphasize the median trend, all collected data of AHN (x_5), AVP (x_6), pH (x_4) and PD (x_7) at each study site under natural (field surveys) or cultivation conditions were transformed into the average over three samplings during different phonological periods before analyses; x_{ai} represents the optimum value of key habitat factor *i* as mentioned above. k_i is the weight coefficient of key habitat factor *i*, then calculated using the formula:

$$k_i = \frac{|a_i| + |b_i|}{\sum_{i=1}^{7}(|a_i| + |b_i|)}, i = 1 \cdots 7 \quad \text{......(4)}$$

Where a_i is the factor loading of key habitat factor *i* in PCA1 (the first principal component); b_i is the factor loading of key habitat factor *i* in PCA2 (the second principal component) (Table 5). The larger the contribution of the key habitat factor is, the higher the weight of the key habitat factor will be.

After calculating, the results of the weight coefficients are: k_1 = 0.141611, k_2 = 0.149163, k_3 = 0.13804, k_4 = 0.143239, k_5 = 0.148563, k_6 = 0.141107, k_7 = 0.138277. Thus, the specific calculation formula was obtained. The values of HNF are shown in Table 6 according to Eq. (3). In order to prove the validity of Eq. (3) as a measure for the degree of similarity of an actual habitat to the optimum habitat, and to explain its mathematical justification, the familiar Proportional Similarity Index (PSI, Feinsinger et al., 1981), which is commonly used in similarity measures in many ecological studies was tested and verified as follows:

$$PSI = 1 - 0.5\sum_{i=1}^{7}|p_i - q_i| = \sum_{i=1}^{7}\min\{p_i, q_i\}, i = 1,2,...7 \quad (5)$$

Here,

$$p_i = \left(\frac{x_i}{\bar{x}_i}\right) \div \left(\sum_{i=1}^{7}\frac{x_i}{\bar{x}_i}\right),$$

$$q_i = \left(\frac{x_{ai}}{\bar{x}_i}\right) \div \left(\sum_{i=1}^{7}\frac{x_{ai}}{\bar{x}_i}\right), i = 1,2,...7 \quad (6)$$

Where $\bar{x}_i$ is the mean of key habitat factor *i*. The calculation results of PSI are shown in Table 6. From Table 6, we obtain that the varied ranges of HNF *F* and PSI are 0.662 ≤ *F* ≤ 0.937 and 0.848 ≤ PSI ≤ 0.983, respectively. Obviously, the varied range of *F* is more extensive than that of PSI; hence *F* better reflects the varied differences of HNF under different habitat conditions on a large-scale eco-geographical distribution.

Seed yield—habitat niche-fitness relationship

Because of the different thermal, hydrodynamic and soil nutrient conditions at different sites in natural conditions, the value of HNF differed among different sites. It was largest in Hongyuan County of Sichuan Province and lowest in Maqu County of Gansu Province (Table 6). The values of HNF under cultivation conditions increased 14.26% on average compared with the values in nature (Table 6). It was found that the lower the value of HNF was in nature, the more it increased under cultivation conditions. For example, the increase in HNF in Maqu County, Gansu Province was nearly 10 times that in Hongyuan County, Sichuan Province (32.628% vs 3.675%, respectively) (Table 6).

Due to different habitat conditions at different sites, the seed yield was also different, and generally low in nature. The seed yield was highest in Hongyuan county, Sichuan Province and lowest in Maqu County, Gansu Province

Table 5. Factor loading matrix of key habitat factors.

The key habitat factor	PCA1 (a_i)	PCA2 (b_i)
Altitude (X_1)	0.8296	-0.18642
Annual >0°C accumulated temperature (X_2)	0.8915	0.1787
Annual average precipitation (X_3)	0.8012	-0.1892
Soil acidity (X_4)	0.0634	0.9643
Soil alkaline hydrolytic nitrogen (X_5)	0.2126	0.8533
Soil available phosphorus (X_6)	0.1949	0.8175
Plant density (X_7)	0.2982	0.6939
Eigenvalue	2.87	2.48
% of variance	47.67	41.20
Cum. % of var.	47.67	88.87

Note: The first principal component (PCA1); The second principal compoment (PCA2).

Table 6. The values of HNF (F) and the Proportional Similarity Index (PSI).

Site	F in nature	F under cultivation conditions	Rate of increase in HNF (%)	PSI in nature	PSI under cultivation conditions
Maqu	0.662	0.878	32.628	0.848	0.954
Hezuo	0.678	-	-	0.856	-
Haiyan	0.708	0.863	21.893	0.871	0.947
Huangzhong	0.731	-	-	0.882	-
Maerkang	0.757	-		0.895	-
Tianzhu	0.775	0.830	7.097	0.904	0.931
Gangcha	0.815	-	-	0.923	-
Aba	0.853	-	-	0.942	-
Ruoergai	0.881	-	-	0.956	-
Menyuan	0.884	0.937	5.995	0.957	0.983
Hongyuan	0.898	0.931	3.675	0.964	0.980

Table 7. Observed and estimated seed yield.

Site	Estimated seed yield value (kg/ha) in nature[b]	Observed seed yield value in nature (kg/ha)[a]	Absolute deviation percent (%)	Estimated seed yield value (kg/ha) under cultivation conditions[b]	Observed seed yield value (kg/ha) under cultivation conditions[a]	Absolute deviation percent (%)
Maqu	54.94	50	9.88	399.41	400.5	0.27
Hezuo	64.98	72.7	10.62	-	-	-
Haiyan	88.08	95.7	7.96	353.87	360.91	1.95
Huangzhong	110.25	110.05	0.18	-	-	-
Maerkang	140.93	140.88	0.04	-	-	-
Tianzhu	166.23	167.42	0.71	269.09	203.55	32.2
Gangcha	236.73	240.6	1.61	-	-	-
Aba	326.05	331.94	1.77	-	-	-
Ruoergai	409.1	418.78	2.31	-	-	-
Menyuan	418.98	428.82	2.29	630.72	710	11.17
Hongyuan	467.88	450.45	3.87	602.89	600	0.48

Note: [a]The data in this column are mean values of three samples; [b]Estimated seed yield per hectare calculated by Eq. (7).

(Table 7). When agricultural technology, especially artificial fertilizer, was put into effect in the transplanting trials, the number of fertile tillers per unit area was enhanced significantly, bringing an increase in seed yield. The mean value of seed yield was 227.94 kg/ha under natural conditions among study sites while under cultivation conditions the mean value of seed yield was 454.99 kg/ha, increasing 99.61% (Table 7). This characteristic exhibited a corresponding trend to that of HNF: the lower the natural seed yield was, the more it increased under cultivation conditions (Table 7).

The value of HNF reflects not only the degree of fitness of the species to its habitat, but also restricts the seed yield. The seed yield is remarkably interrelated with HNF, but it is not a linear relationship. Taking HNF as the regressor, the relationship was fitted by least squares as log-log regressions of seed yield on HNF, as follows:

$$\ln Y = 6.904 + 7.025 \ln F \qquad (7)$$

Where Y is the estimated seed yield per hectare at F level of HNF. R^2, adjusted R^2 and the standard error of the estimate reached 0.987, 0.986 and 0.094, respectively. The estimated seed yield was calculated by Eq. (7), and observed values are shown in Table 7. The estimated seed yield was well consistent with the observed data, and the average value of the absolute deviation percent was 5.46%, demonstrating the validity of the model in predicting seed yield.

DISCUSSION

Key habitat factors

Much of the research conducted in ecological science is devoted to analyzing species–environment relationships (Harper, 1977), and this has produced a lot of approaches that relied entirely on the choice of ecological factors (Wang, 1990; Retuerto and Carballeira, 2004). Therefore, selecting key factors, which describe an actual habitat state, plays a crucial role in the content of the relationship between species and environment. In fact, the following habitat factors: sunlight, temperature, geographic range, soil water content, and soil nutrition may be closely related to the growth period of crops. In our study the habitat factors associated with seed yield were condensed into seven by Pearson Correlation and Partial Correlation Analyses, a comparison of alternative methods to identify the most important variables influencing seed yield. This way, the effect of interactions between different key habitat factors can be accounted for. The seven key habitat factors not only contained climatic factors such as AT and AP, the site factor AL, edaphic factors such as AHN, AVP and pH, but also plant density (PD). The components of key habitat factors included in the HNF model have the advantage of integrating geographic data with plant performance. Moreover, they also meet the needs of the soil-plant-atmosphere continuum theory. This suggests that the core of construction of the HNF model should be focused on the seven key habitat factors. The HNF model containing these key habitat factors makes it possible to evaluate the adaptive extent of *M. sikkimensis* to different habitat conditions in a large-scale eco-geographical study.

Construction of habitat niche-fitness model

An apparent supply–demand relationship exists between a realized habitat and species' optimum habitat requirements during the *M. sikkimensis* growth cycle. The niche theory as a kernel of modern ecology provides a sound theoretical background for HNF (Hutchinson, 1957; Li and Lin, 1997; Lin and Li, 1998; Buggeman and O'Nuallain, 2000). The characters of the habitats in reality are different from its optimum niche in some aspects. If an actual habitat or a modified habitat which suited the species existed, the population would increase, exhibiting an increase of yield; otherwise, it would decrease, resulting in a decrease of yield. That tallies with the concept of HNF. Normally, when the actual habitat state changes on a large scale, the value of HNF needs a wide distribution on the subset [0, 1]. By taking *M. sikkimensis* as the object, Hutchinson's (1957) niche concept of *n*-dimensional super-volume was extended; the HNF for *M. sikkimensis* is defined as the degree of similarity between the supply of an actual habitat and the requirement for the optimum habitat, and is a synthesis of key habitat factors to describe a habitat state. The mathematical model for HNF in Eq. (3), which is the degree of similarity defined in n-dimensional supervolume in essence, first calculated the relative degree of similarity between an actual habitat state and the optimum habitat requirement. In fact, the importance of various key habitat factors to *M. sikkimensis* is different. Considering the actual conditions of unequal weight among the key habitat factors, the weights of key habitat factors as HNF model parameters play a crucial role in model usability. Then, the weight determination of key habitat factors was integrated with factor loadings derived from a Principal Component Analysis (PCA) in this study. This method as a measure for the degree of similarity of an actual habitat to the optimum habitat is a new improvement compared with the Proportional Similarity Index (Feinsinger et al., 1981), which is commonly used in similarity measures in many ecological studies. The values of HNF obtained from Eq. (3) have wider distribution, i.e. $0.662 \leq F \leq 0.937$, compared with those obtained from the Proportional Similarity Index (PSI), $0.848 \leq \text{PSI} \leq 0.983$. Therefore, the HNF model is superior to the Proportional Similarity Index. From the point view of agro-ecology, the HNF is a new concept which indicates a description of the adaptability of the species to its habitat. Our results suggested that HNF be a new model to evaluate the adaptive extent of *M. sikkimensis* on a large-scale eco-geographical distribution.

The seed yield prediction model

Many yield prediction models have been developed since the 1960s (Dahl, 1963; Duncan and Hesketh, 1968; Rosensweig, 1968; Murphy, 1970; Duncan and Woodmansee, 1975; Seligman and Van, 1989; Wang, 1990; Scian and Bouza, 2005). Their performance requires a lot of parameters relating to plant physiological processes, such as photosynthesis, assimilation and respiration (which respond to climatic conditions), soil moisture, and fertilizer application, but determining these parameters is quite difficult. Using HNF as a surrogate for composite environment factors to establish the seed yield prediction model is a new approach and has proved to be effective. Our results show that the simulation agrees well with observed seed yield, which means that the model has a high predictive power for seed yield. Compared with traditional yield prediction models, the model based on HNF provides a new approach to predict seed yield accurately.

Upper limit of HNF and seed yield, threshold value of HNF and limit seed yield

Studying HNF variation in response to uncontrollable and controllable key habitat factors provides a good estimate of fitness variance. While each controllable key habitat factor alone can be experimentally manipulated to study its influence on fitness, the relative importance and potential impacts of different factors are very hard to investigate experimentally (Retuerto and Carballeira, 2004). Here, we attempted to include seven key habitat factors simultaneously in an analysis. The effects of controllable key habitat factors on the survival and growth of *M. sikkimensis* were examined under cultivation condition at five sites selected from eleven field sites. In this study, the trials under cultivation indicated that agricultural measures significantly increased the value of HNF and seed yield, which implies that agricultural measures debugged controllable key habitat factors to approach their optimum values resulting in habitat modification. In general, the key habitat factors explaining variability in HNF are technological change, including moderate fertilization, improved management practices and disease control, as well as other human interventions aimed at increasing seed yield. However, even if technological innovations in cultivation are optimized, the increase in HNF cannot be infinite. Therefore, each site has its upper limit of HNF. Eq. (3) is used to forecast this limit when all controllable key habitat factors reach their optimum values, and the results are shown in Table 8. The seed yield corresponding to the upper limit of HNF for each study site is forecasted using Eq. (7); results are listed in Table 8 as upper limit of seed yield. The maximal upper limit of seed yield is predicted to be 821.98 kg/ha at Haiyan County, Qinghai Province, with the minimal upper limit of seed yield being 396.22 kg/ha at Maerkang County, Sichuan Province. These results suggest that Haiyan County, Qinghai Province has the best productive potential among the eleven study sites. Understanding the control of HNF by cultivation technology would result in better adaptation for plants to habitat conditions and a greater seed yield for *M. sikkimensis.* The upper limit of seed yield should be obtainable under optimized agricultural technology when the effects of the relationship between HNF and seed yield are understood and managed accordingly.

When $F = 0.75$, a prediction interval with a 95% confidence interval for seed yield (kg/ha) by Eq. (7) is [113.91, 153.12] where the seed yield is acceptable because the seed yield per kg was priced at 3.63 dollars. The membership of a certain habitat, i.e. whether or not a certain area is favorable for *M. sikkimensis*, is determined by a threshold value defined as F=0.75. If this so-called cutpoint is exceeded, the habitat shows an acceptable seed yield and vice versa (Table 9). Therefore, computing HNF can become a decision making tool to tell the farmers whether *M. sikkimensis* can grow at a target site or not. An appropriate HNF value will make the plants thrive, but an excessively low value would result in physiological malfunction to the plants and even make them die off. Considering the commercial and biological seed yield requirements of *M. sikkimensis*, a HNF membership grade matrix was constructed to specify the distribution patterns of *M. sikkimensis* in different HNF intervals (Table 9).

Table 8. Upper limit of HNF and seed yield (kg/ha) for *M. sikkimensis* among study sites.

Site	Upper limit of HNF	Upper limit of seed yield (kg/ha)
Maerkang	0.877	396.22
Huangzhong	0.896	460.61
Aba	0.907	501.83
Hezuo	0.917	542.02
Maqu	0.943	659.65
Ruoergai	0.944	664.58
Hongyuan	0.949	689.71
Menyuan	0.956	726.25
Gangcha	0.965	775.66
Tianzhu	0.969	798.53
Haiyan	0.973	821.98

From the theoretical point of view, when all values of the key habitat factors reach the optimum, then value of HNF attains the maximum, that is F=1, and the corresponding seed yield goes by the name of the limit seed yield. This reaches 996.25 kg/ha as forecast by our Eq. (7). This result coincides with the theoretical seed yield of 984 kg/ha extrapolated by An and Ma (2002) from the average seed yield per plant in pot experiments at 2000 in Menyuan County, Qinhai Province.

The aims of the HNF model and seed yield prediction model are to help farmers correctly select suitable target sites and create meaningful summaries of site-specific management information. They can provide good answers to farmers' questions relating to utilization and development of *M. sikkimensis* in the following aspects: whether introduction of *M. sikkimensis* in a particular area is suitable or not, judged by the HNF (>0.75) and further judged by the seed yield in nature and the potential seed yield under cultivation conditions. Seed yield is tightly related to the value of HNF. Therefore, we can enhance HNF through rational agricultural technology and then improve seed yield. For example, we can attain the goal of improving the HNF for *M. sikkimensis* by forecasting each index of uncontrollable key factors, ensuring rational cultivation technology, and ultimately increasing seed yield. Further improvements in these models, such as combining them with a geographic information system (GIS) database to compile maps on HNF and seed yield prediction, are envisaged to improve ease of application.

Table 9. The grades of HNF for *M. sikkimensis* and distribution patterns in different HNF interval.

Grades	HNF interval	Predictive seed yield interval (kg/ha)	Distribution zone	Suitability and biological performance
1	0.9-1	476.20-998.25	The core area	Optimal. It thrives and has high reproduction.
2	0.75-0.9	132.30-476.20	Appropriate eco-environmental zone	Suitable. It grows and reproduces satisfactorily.
3	0.55-0.75	14.97-132.30	Restricted zone	Quasi suitable. Its growth is restrained and reproductive capacity declines.
4	0.40-0.55	1.60-14.97	Marginal zone	Adverse. Its growth and survivor are abnormal, and reproduction is impossible.
5	<0.40	<1.60	Survival forbidden zone	Unsuitable survival.

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微孔草（*Microula sikkimensis*）生境生態棲位適宜度與草籽產量關係的研究

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微孔草（*M. sikkimensis*）是隸屬於紫草科微孔草屬的兩年生草本植物，主要分佈于青藏高原東緣的退化草甸和草原，作為新興的在地油料作物種，其在醫藥、保健、飼草料等方面均具有多方面的新應用，因此，在青藏高原日益退化的高山草甸和草原放牧生態系統中，利用微孔草草籽生產，建立可持續的生產生態系統，不失為一種既取得生產效益又利於生態恢復的理性選擇之一。自 1994 年 4 月至 2004 年 9 月，先後在青藏高原東緣的 3 省 11 縣進行了大範圍的野外調查和移栽試驗，收集了各研究位點的大量生境參數，通過相關與偏相關分析篩選出描述生境的七個關鍵生境因子。引入生態棲位理論，定義微孔草生境生態棲位適宜度是現實生境供給與微孔草對生境的最適需求之間的相似程度，在對各關鍵生境因子對生境生態棲位適宜度的權重進行界定的基礎上，構建了微孔草生境生態棲位適宜度模型，對野外調查和移栽試驗的計算結果表明：生境生態棲位適宜度較好地反映了不同研究位點的生境差異。在推薦的農藝措施下，因改善了生境促進了微孔草生長，各移栽試驗點的生境生態棲位適宜度都有不同程度地提升，平均提升率達 14.26%，亦促使草籽產量平均增長 99.61%；生境生態棲位適宜度作為諸多環境因子的綜合指標與草籽產量間存在顯著的雙對數回歸關係，經實驗數據驗證，觀測記錄的草籽產量與該回歸關係模擬估計值之間的平均絕對離差百分數是 5.46%，意味著該回歸方程在大尺度預測草籽產量上的可用性，並且以生境生態棲位適宜度為自變量建立草籽產量預測模型的方法，比之於傳統產量預測模型而言，具簡便準確之利，是一種新的嘗試。通常農藝措施通過調控可控關鍵生境因子逼近最優值，實現生境改善，從而提高了生境生態棲位適宜度值和草籽產量，然而這種提升力並不是無限的，因此每個位點都有其生境生態棲位適宜度上限值和相應的草籽產量上限。生境生態棲位適宜度模型、草籽產量預測模型及其研究結果對選擇微孔草適宜生長區，進行栽培、生產、管理具有指導意義。

關鍵詞：生境生態棲位適宜度 (HNF)；微孔草 (*Microula sikkimensis*)；草籽產量；青藏高原。