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SPECIES DIVERSITY, VEGETATION PATTERN AND CONSERVATION OF *GENTIANA MACROPHYLLA* PALL. COMMUNITIES IN DONGLING MOUNTAIN MEADOW, BEIJING, CHINA

SEHRISH SADIA[1*], JIN-TUN ZHANG[1*], AKASH TARIQ[2,3], XIAOHANG BAI[1], ARSHAD ALI SHEDAYI[4,5], KE CAO[1], PARAS MAZARI[1], SIDRA ASLAM[1] AND LATIF AHMAD[1]

[1]*College of Life Sciences, Beijing Normal University, Beijing-100875, China*
[2]*Key Laboratory of Mountain Ecological Restoration and Bioresource Utilization & Ecological Restoration Biodiversity Conservation Key Laboratory of Sichuan Province, Chengdu Institute of Biology, Chinese Academy of Sciences, Chengdu-610041, People's Republic of China*
[3]*University of Chinese Academy of Sciences, Beijing-100039, People's Republic of China*
[4]*Department of Biological Sciences, Karakoram International University, Gilgit, Pakistan*
[5]*Institute of Geographic Sciences and Natural Resource Research, Chinese Academy of Sciences, Beijing-100101, China*
[*]*Corresponding author (s): zhangjt@bnu.edu.cn; sehrish@mail.bnu.edu.cnPh.No. 008613520821020*

Abstract

Gentiana macrophylla, native to mountainous areas of Central and Southern Asia, is most popular remedy for rheumatism and pains in Traditional Chinese Medicine with an extensive demand in local market. Our study aimed to classify *G. macrophylla* communities and to find out the impact of topographic and soil factors on their diversity and distribution in Dongling mountain meadow, Beijing, China. Seventy five samples in 15 transects separated by 50m distance in altitude along an elevation gradient (1592-2298m) were established by quadrate method. TWINSAPN and CCA were used for classification and ordination, respectively. Six diversity indices (Species richness, Shannon-Weiner heterogeneity, Simpson's index, Hill's index, Pielou evenness and McIntosh evenness) were used to analyze the pattern of species diversity and polynomial regression analysis was used to establish their relationship with environmental variables. TWINSPAN classified *G. macrophylla* communities into 8 types and CCA indicated that soil pH, soil temperature, soil type, disturbance, total N, total K, Mg and Zn were significantly related to these communities. Elevation was the most significant factor that affecting the diversity and distribution of *G. macrophylla* communities. Significant effect of environment, topography and disturbance to meadow communities of *G. macrophylla* highly suggests some important measures such as uprooting restriction, tourism limitation in meadow area, monitoring of functional diversity, fertilization, irrigation, cloning and cultivation to protect and conserve it and its communities to be used in medicine industry.

Key words: Ecology, Medicinal plants, Mountain, Gentiana, Qin-Jiao.

Introduction

Mainland China has a very large phytodiversity. However, to cope with a huge population, industrialization and cultivation caused a large number of species to disappear from plains and low lands. But mountainous areas still have a large number of those species which need to be preserved and conserved keeping in view the increasing trend of mountainous tourism (Tang *et al.*, 2006; Zhang & Dong, 2009; Monz *et al.*, 2010). Understanding vegetation pattern and diversity plays a crucial role for the conservation of mountainous flora (Zhang *et al.*, 2013).

Mountain meadows are one of the most important parts of mountainous ecosystem having unique type of grassland above the timberline. They provide various ecosystem services such as living place for wildlife, livestock forage and natural recreational spots etc. (Attore *et al.*, 2013; Zhang *et al.*, 2015). People are attracted towards mountains to enjoy and explore the natural beauty (Pickering & Buckley, 2003; Cheng *et al.*, 2005; Monz *et al.*, 2010). With the increase of this mania, interruption and disintegration of mountainous areas is increasing day by day. Most severe disturbance is caused to meadows due to fragile life form of herbaceous plants (Zhang & Dong, 2009).

Most of the Chinese mountain meadows are present in the west of country (Kunlun Mountains, Qilian Mountains, Tibetan plateau and Tianshan Mountains). While northern China has fewest of total among which mostly are distributed on Dongling mountain nature reserve bordering Beijing in West (Zhang & Dong, 2009; Zhang *et al.*, 2015). Nature areas of Beijing (capital city of China) are very limited (Xu & Zhang, 2008). Therefore, these natural areas especially meadows are of immense importance as ecological barrier between this highly populated city and its citizens (Xu *et al.*, 2007).

Gentiana macrophylla Pall., large leaf gentian, is a highly used important medicinal plant in China, and Dongling Mountain is the only habitat of this species in Beijing. Qin-Jiao, a famous Chinese herbal remedy, mainly refers to the roots of *G. macrophylla*. It is used to treat the bone fever, stroke, pains, rheumatism, jaundice and heat deficiency syndrome (Cai *et al.*, 2010). This plant and its communities are at possible and potent risk of extinction due to heavy digging of its roots to be used in medicine industry. So the conservation of *G. macrophylla* is highly needed. Distribution and Diversity of medicinal plants is affected by environmental gradients (Zilliox & Gosselin, 2014), also the pattern of changes of species diversity provides the basis for the conservation of natural reserves (Muhumuza & Byarugaba, 2009).

Previously study of plant resources (He *et al.,* 1992), functional diversity (Zhang *et al.,* 2015), flora and vegetation ecology (Xiang & Zhang, 2009) and specifically, study of distribution of medicinal plant communities in relation to environment and topography (Nyobe *et al.,* 2012) were carried out on Dongling Mountain. A lot of studies are carried out on medicinal properties (Wu *et al.,* 2011), phytochemical analysis (Olennikov *et al.,* 2015) and pharmacognostic evaluation (Ma *et al.,* 2010; Wei *et al.,* 2006) of *G. macrophylla* but its ecological studies were very few. To the best of our knowledge, this is first ecological study of *G. macrophylla* communities to be carried out in Beijing, China. The objectives of this study were; (1) to carry out the classification and ordination of *G. macrophylla* communities from Dongling mountain meadow, (2) to find out the influence of environmental factors on the distribution and diversity of *G. macrophylla* communities, and (3) to evaluate soil minerals concentration and its influences on altitudinal distribution of *G. macrophylla*, (4) to suggest the measures for conservation and protection of *G. macrophylla* communities on the basis of our results.

Materials and Methods

Study area: The Dongling Mountain is located at 40°00'-40°05' N, 115°26'-115°40' E. It is a part of Taihang Mountain range in Beijing, China and an extension of Xiaowutai Mountain, Shanxi, China (Liu *et al.,* 2010). It has a temperate semi humid monsoon climate with an annual mean temperature of 7°C, annual average precipitation of 500-650mm (Zhang *et al.,* 2015) and relative humidity of 66°C (Liu *et al.,* 2010). Monthly mean temperature in coldest and hottest month is -7.8°C and 21.1°C, respectively. Various soil types such as cinnamon soil, mountain cinnamon soil, brown forest soil and mountain meadow soil can be found in this mountain range (Huo, 1989). Elevation of Dongling Mountain is 800-2301m. Meadows occur in the range of 1600 to 2301m. Meadow area is the main tourist site on Dongling Mountain (Zhang *et al.,* 2012).

Data collection and sampling: On the basis of general survey of *G. macrophylla*, 15 sampling transects were set along elevation gradient from 1592-2298m in the area of *G. macrophylla* distribution. Five samples were collected from each transect by using quadrats of 1 m × 1 msize (standard size for meadow). Species and environmental data were recorded in each quadrat.

Species data: Complete plant list was made from each quadrat. Cover and height of plant species were measured in each quadrat. Cover was estimated visually and plant height was measured by using a ruler. A total of 85 plant species were recorded in 75 quadrats.

Environmental data: Altitude, slope, slope aspect, soil pH, soil type, Soil temperature and disturbance intensity were recorded as environmental factors for each quadrat. Altitude, slope and aspect, soil pH, soil temperature were measured by using GPS, Compass meter, pH meter and thermometer, respectively (Zhang, 2004; Zhang *et al.,* 2013). Soil type was recorded by rope test method (Nyobe *et al.*, 2014). Standardization of aspect measurements was carried out by giving them classes form 1-8 as follows: 1 (333.7-22.5°), 2(22.6-67.5°), 3(292.6-337.5°), 4(67.6-112.5°), 5(247.6-292.5°), 6(112.6-157.5°), 7(202.6-247.5°). Disturbance intensity was noted on the basis of number of tourists, distance from nearest road and human population, garbage quantity, grazing and trampling (Zhang *et al.,* 2013). It was evaluated on a scale of 1-5 as follows; 1 (no obvious disturbance), 2 (weak disturbance), 3 (medium disturbance), 4 (heavy disturbance), 5 (very heavy disturbance).

Soil sampling: Three soil samples from 3 quadrats out of total 5 in each transect were collected. Soil samples were collected by shovel at the depth of 20cm. Then were mixed to make one sample and put into zipper bags with labels before taking to lab. Finally there were 15 samples for 15 transects.

Soil processing and analysis: Moist soil samples were taken to lab. The rope test was used to check the soil type. Then soil was shade dried for several days at room temperature. Dried soil was ground with pestle and mortar, sieved with 0.2mm mesh size sieve and weighed by using electronic balance to make soil solution. Total nitrogen, total phosphorus, total potassium, magnesium and zinc concentration was determined by using inductively coupled plasma emission spectrophotometer (Philips Innovation Services, 2013; Hou, 2000), visible spectrophotometer (Schmid, 2001) and Atomic absorption spectrophotometer (Ata, 2015). LY/T 1228-1999, LY/T 1232-1999, LY/T 1234/1999, LY/T 296-1995, GB/T 17138-1997 test standards were used for total N, total P, total K, Mg and Zn respectively.

Data analysis: Species and environmental data matrix was made for multivariate analysis. Importance values (IV) of 85 plant species in 75 quadrats were used to make species data matrix. It was calculated by using following formula:

$$IV= (\text{relative cover}+ \text{relative height})/2$$

The environmental data matrix consisted of values of 7 environmental variables (Altitude, slope and aspect, soil pH, soil temperature, soil type and disturbance) and 5 soil minerals (total N, total P, total K, Mg and Zn).

WINTWINS and CANOCO 5 software (TerBraak & Smilauer, 2002; Zhang & Shao, 2015) were used for the calculation of TWINSPAN (for classification of *G. macrophylla* communities) and CCA (to analyze their relationship with environmental variables).

Six species diversity indices, one for species richness, three for species heterogeneity and two for species evenness were used to calculate diversity values as follows:

Species number (as richness index): D=S (Zhang, 2004).

Shannon-Weiner heterogeneity index: $H' = -\sum_{i=1}^{s} Pi \log(Pi)$ (Karami *et al.*, 2015; Mutiso *et al.*, 2015).

Simpsons Index: $D = 1 - \sum_{i=1}^{s} Pi^2$ (Bibi & Ali, 2013).

Hill's heterogeneity index: N2= $\frac{1}{\sum_{i=1}^{s} \frac{Ni(Ni-1)}{N(N-1)}}$ (Contoli & Luiselli, 2015).

Pielou evenness index: E1= *H'*/ln(S) (Jost, 2010; Neelamegam *et al.*, 2015).

McIntosh evenness index:
E=$([N - \sqrt{(\sum ni^2)}]) / ([N - (N/\sqrt{(S)})])$
(Lexerod & Eid, 2006).

where *Pi* is the relative importance value of species *i*, *Ni* the importance value of species *i*, *N* the sum of importance values for all species in a quadrat, and *S* the species number present in a quadrat.

Polynomial Regression analysis was carried out to create relationship between species diversity and environmental and topographic variables.

Results

Classification of *G. macrophylla* communities: TWINSPAN classified 75 quadrats into 8 groups representing 8 communities of *G. macrophylla* (Fig. 1).

1. Comm. I *Artimisa sacrorum* + *Gentiana macrophylla* + *Carex subpediformis*
2. Comm. II *Carex subpediformis* + *Agrimonia pilosa* + *Sanguisorba officinalis* + *Gentiana macrophylla*
3. Comm. III *Carex* sp. + *Agrimonia pilosa* + *Gentiana macrophylla*
4. Comm. IV *Carex* sp. + *Gentiana macrophylla* + *Sanguisorba officinalis*
5. Comm. V *Gentiana macrophylla* + *Kobresia myosuoides*
6. Comm.VI *Kobresia myosuoides* + *Gentiana macrophylla* + *Plantago asiatica*
7. Comm. VII *Gentiana macrophylla* + *Potentilla chinensis* + *Leontopodium leontopodioides*
8. Comm. VIII *Caragana arborescens* + *Gentiana macrophylla* + *Kobresia myosuoides*

Composition and characteristics of *G. macrophylla* are given in Table 1.

Name of *G.macrophylla* communities are following;

Ordination analysis: Canonical correspondence analysis was carried out for 75 quadrats and 12 environmental variables (7 topographic variables and 5 Soil minerals). The Monte Carlo permutation test showed that eigenvalues for the entire canonical axis were significant (p<0.001). Eigenvalues for the first four CCA axes were 0.387, 0.220, 0.175 and 0.142; the species-environment correlations for the CCA axes were 0.946, 0.869, 0.822 and 0.826. Distribution of *G. macrophylla* communities was significantly related with elevation, soil pH, soil type, soil temperature, disturbance, total N, total P, total K and Mg content in soil.

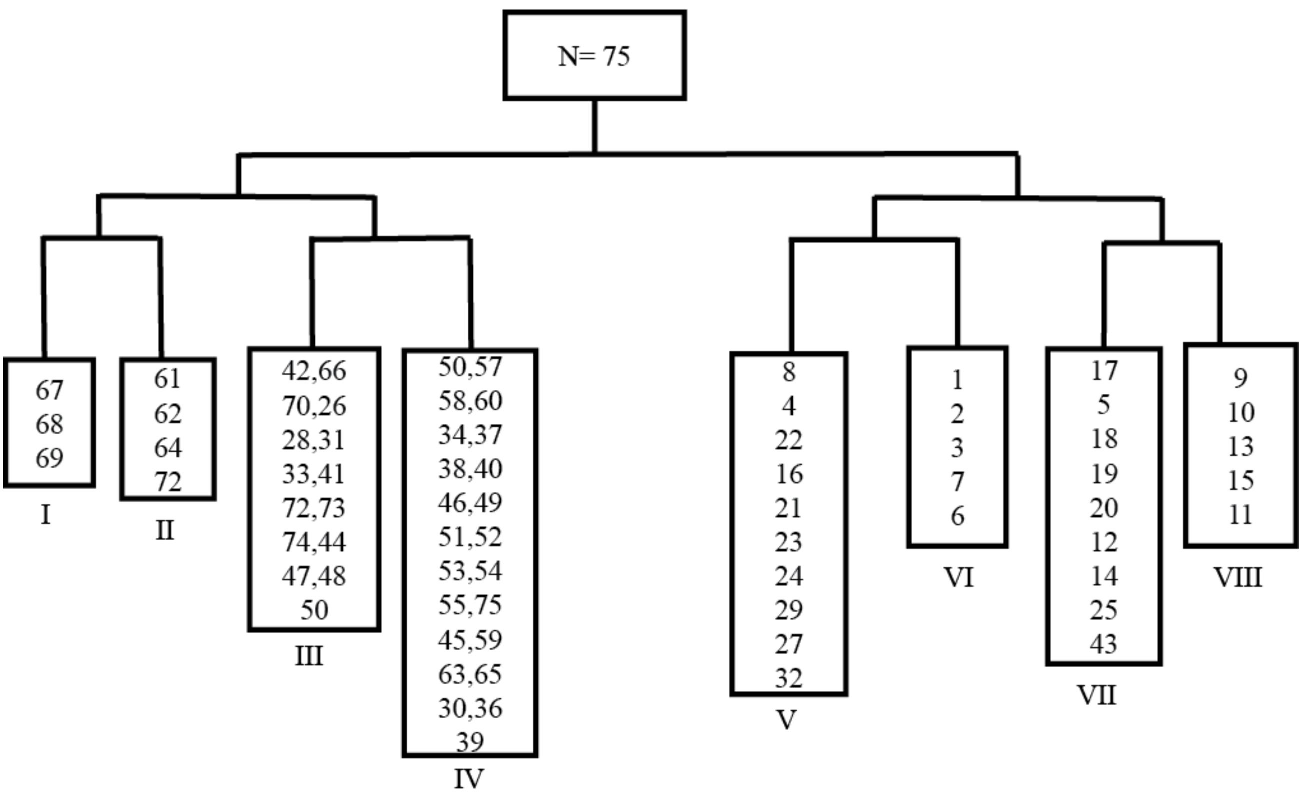

Fig. 1. Dendrogram of TWINSPAN results for 75 quadrats of plant communities in Dongling mountain meadow. Roman numerals I-VIII represents eight communities of *G. macrophylla* and Arabic numerals represent the quadrat numbers (samples). Altitude increases from right to left.

Table 1. Attributes of *G. macrophylla* communities in Dongling mountain meadow.

Community	Elevation (m)	Slope (°)	Total community cover (%)	Cover of *G. macrophylla* (%)	IV of *G. macrophylla* (%)	Soil pH	Disturbance	Other Common species
I (sample 67, 68, 69)	1645-1649	10-15	94.07	15.7	8.456	6.02-6.5	4-5	*Scabiosa atropurea, Potentilla supina*
II (Sample 61, 62, 64, 71)	1604-1696	3-15	84.9	5.125	6.902	5.99-6.39	3-5	*Plantago asiatica, Gallium verum, Scabiosa atropurea etc.*
III (Sample 42, 66, 70, 26, 28,31, 33, 41, 72, 73, 74, 44, 47, 48, 50)	1602-2054	3- 26	81.83	8.58	10.05	5.5-6.86	2-5	*Sanguisorba officinalis, Callistephus chinensis, Dendranthema chanetii, Plantago asiatica*
IV (Sample 56, 57, 58, 60, 34, 37, 38, 40, 46, 49, 51, 52, 53, 54, 55, 75, 45, 59, 63, 65, 30, 36, 39)	1757-2046	3-25	82.39	11.435	10.845	5.45-6.77	1-5	*Agrimonia pilosa, Callistephus chinensis, Scabiosa atropurea, Dendranthema chanetii*
V (Sample 8, 4, 22, 16, 21, 23, 24, 29, 27, 32,35)	2001- 2292	2-30	63.73	12.272	17.21	5.91-7.2	1-5	*Plantago asiatica, Callistephus chinensis, Sanguisorba officinalis, Potentilla chinensis*
VI (Sample 1, 2, 3, 7, 6)	2251-2298	3-27	80.2	6.25	10.941	6.55-7.12	1-2	*Anemone cathyensis, Potentilla chinensis*
VII (Sample 17, 5, 18, 19, 20, 12, 14, 25, 43)	1901-2291	1-8	57.56	6.777	11.816	5.79-6.75	1-3	*Plantago asiatica, Sanguisorba officinalis*
VIII (Sample 9, 10, 13, 15, 11)	2187-225.5-6,8657	3-20	59.8	7.6	11.388	6.19-6.79	1-2	*Dendranthema chanetii, Taraxacum mongolicum, Sanguisorba officinalis*

Table 2. Correlation co-efficients between environmental variables in *G. macrophylla* communities in Dongling mountain meadow, Beijing, China.

	Elevation (m)	Slope (°)	Aspect	Soil pH	Soil type	Soil temp. (°C)	Disturbance	Total N (g)	Total P (g)	Total K (g)	Mg (g)	Zn (mg)
Elevation	1											
Slope	-0.011	1										
Aspect	-0.0622	0.0794	1									
Soil pH	0.4351***	-0.0449	0.0091	1								
Soil type	-0.0688	-0.2242*	-0.0459	-0.1668	1							
Soil temp.	-0.6532***	0.2188*	0.173	-0.2546*	0.0143	1						
Disturbance	-0.6109***	-0.0102	-0.0316	-0.2043	-0.1454	0.2755*	1					
Total N	0.0303	0.3336**	0.1896	-0.2104	0.252*	0.122	-0.2727*	1				
Total P	0.4414***	0.2792*	-0.0222	0.0264	-0.2307*	-0.4118***	-0.0128	-0.1035	1			
Total K	0.0932	-0.2901**	0.0238	-0.2238*	-0.0484	-0.1515	-0.2308*	-0.3026**	0.0716	1		
Mg	0.088	0.0042	-0.0244	-0.1205	-0.2405*	0.0775	-0.2781*	-0.2615*	-0.0992	0.7779	1	
Zn	-0.1224	0.1767	0.1776	-0.3321**	0.2987**	0.0384	0.0886	0.586***	-0.1383	-0.0892	-0.219*	1

Legend: *p<0.05, **p<0.01, ***p<0.001 where*, ** and *** means significant, more significant, most significant, respectively

Table 3. Interdataset correlation co-efficients of environmental variables with CCA axes in *G. macrophylla* communities in Dongling mountain meadow, Beijing, China.

	Axis 1	Axis 2	Axis 3	Axis 4
Elevation	0.9051***	-0.122	-0.0958	-0.0885
Slope	-0.0203	-0.0998	-0.1044	0.3018**
Aspect	-0.0692	0.0065	-0.0099	0.0642
Soil pH	0.4906***	-0.0423	0.3852***	-0.0699
Soil type	-0.1262	-0.4357***	-0.2974**	0.2437*
Soil temperature	-0.5019***	0.024	0.3776***	0.4387***
Disturbance	-0.4408***	0.6053***	-0.106	0.1599
Total N	-0.6419***	-0.0189	-0.2247*	0.1715
Total P	-0.1966	0.6865***	0.301**	-0.1171
Total K	0.2448*	0.2097	-0.4052***	-0.4387***
Mg	0.3105**	-0.0377	-0.1522	-0.5062***
Zn	-0.0031	0.2622*	-0.3938***	0.3586***

Legend: *p<0.05, **p<0.01, ***p<0.001 where*, ** and *** means significant, more significant, significant, respectively

Table 4. Diversity indices of each vegetation community of *G. macrophylla* communities in Dongling mountain meadow, Beijing, China.

	Species richness	Shannon-weiner index	Simpsons index	Hill's number	Pielou evenness	McIntosh evenness
Comm I	13.667	1.011	0.114	44.378	0.388	1.084
Comm II	14.750	1.111	0.079	58.306	0.413	1.091
Comm III	13.067	1.029	0.107	45.907	0.402	1.094
Comm IV	13.565	1.042	0.103	46.872	0.400	1.093
Comm V	10.273	0.938	0.124	33.259	0.405	1.104
Comm VI	13.800	1.059	0.101	50.899	0.402	1.115
Comm VII	10.222	0.949	0.122	35.889	0.412	1.103
Comm VIII	11.000	0.971	0.116	31.162	0.406	1.108

Correlation coefficient among environmental variables indicated that elevation was significantly correlated with soil pH, soil temperature, disturbance and total phosphorus. Total Nitrogen was significantly correlated with total Mg and Zn but showed insignificant correlation with total phosphorus (Table 2). First CCA axis was significantly correlated with Elevation, soil temperature, soil pH, disturbance and total N. Correlation of first axis with elevation was positive while with soil temperature, disturbance and total N was negative. So first axis was comprehensively dominated by elevation gradient with an eigenvalue of 0.387. Second CCA axis was significantly correlated with disturbance, soil type, total phosphorus and Zinc (Table 3). Elevation among topographic and total nitrogen among soil variables were dominant factors to define the distribution of *G. macrophylla* communities on Dongling mountain meadow (Fig. 2; Table 3).

Species diversity: Variation in species diversity in *G. macrophylla* communities was observed. Species number varied from 10 to 15, Shannon-Weiner index varied from 0.938 to 1.111, Simpson index varied from 0.079 to 0.124, Hills number varied from 31.162 to 58.306, Pielou evenness varied from 0.388 to 0.413 and McIntosh evenness varied from 1.084 to 1.115 among communities of *G. macrophylla* (Table 4).

Species richness, heterogeneity and evenness were significantly related to elevation and soil temperature (Fig. 3A-F, Fig. 4A-F).

Discussion

G. macrophylla dominated communities showed variation in structure and composition. Eight communities classified by TWINSPAN are representative of *G. macrophylla* population in Beijing. This classification schemes is according to Chinese vegetation classification system (Wu, 1980; Zhang *et al.*, 2013). Indicator species at different divisions of TWINSPAN such as *Agrimonia pilosa, Carex* sp., *Carex subpediformis, Potentilla supina, Kobresia myosuoides, Callistephus chinensis, Leontopodium leontopodioides, Deyeuxia arundinacea, Sanguisorba officinalis, Papavern udicaule, Potentilla chinensis* dominated the *G. macrophylla* communities and they played significant role in structure of community in at least one community type (Zhang, 2002). Highest percentage cover of *G. macrophylla* was found in Community II, whereas if we look both at cover and importance value then Community V is dominated by *G. macrophylla*. As this community is present in the elevation range of 2001- 2292m, it indicates that *G. macrophylla* is the species of higher altitudes. Xu and Zhang (2008) used the same method of classification for *Acanthopanax senticosus* communities in Dongling Mountain. Their results indicated 9 *Acanthopanax senticosus* communities all preferred to grow at high altitude (Xu & Zhang, 2008). Similar studies were also carried out at other mountains e.g. Wutai mountain (Zhang & Mi, 2007), Baihua mountain (Zhang *et al.*, 2013), Song mountain (Suriguga *et al.*, 2010).

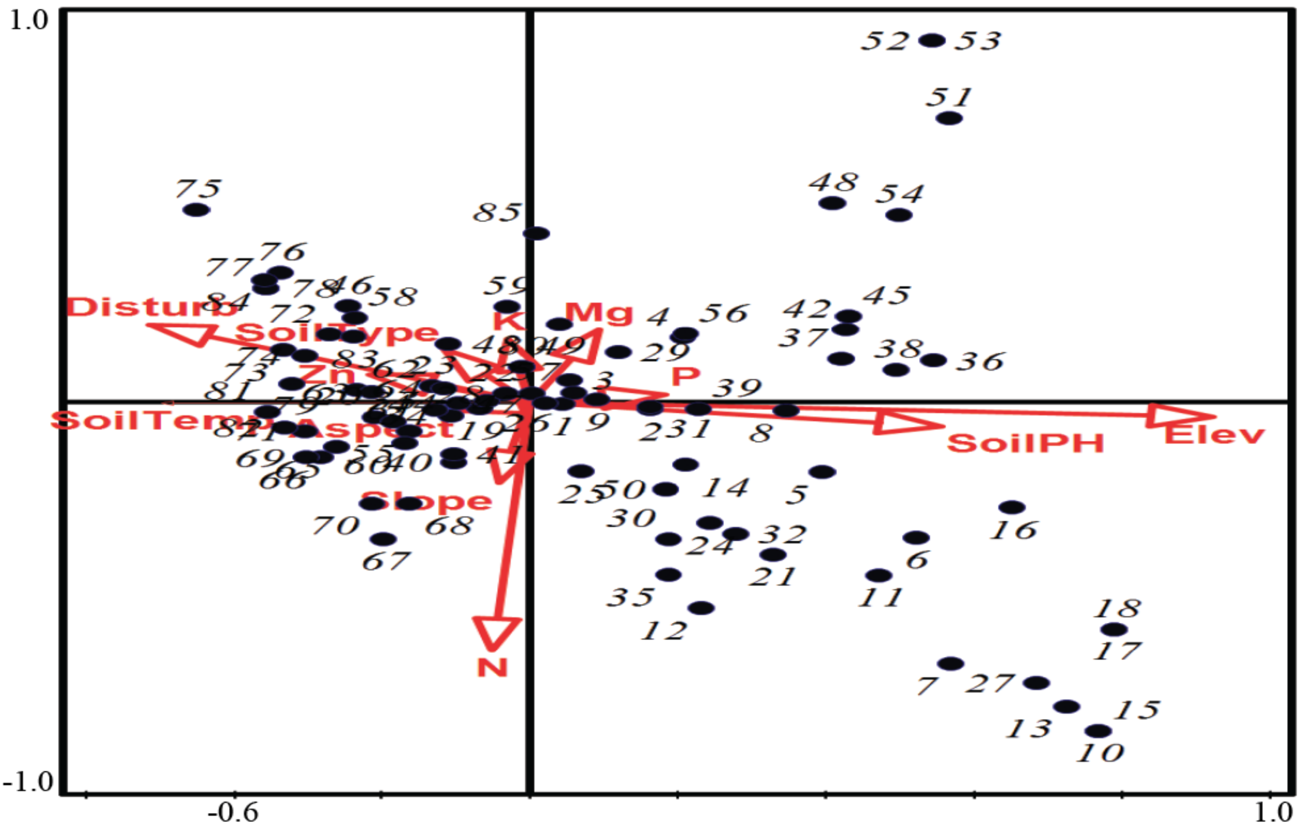

Fig. 2. CCA ordination biplot of 85 species and 12 environmental variables of *G. macrophylla* communities in Dongling mountainmeadow, Beijing, China. The number refers to quadrats.

Ordination analysis indicated the importance of topographic and soil variables to *G. macrophylla* communities just as to *Glycyrrhizaauralensis* communities in Northern China studied by Zhang *et al.* (2011). Placement of *G. macrophylla* communities at different locations in CCA plot clearly indicates the community-environment correlation (TerBraak & Smilauer, 2002; Brinkmann *et al.*, 2009). As first CCA axis was dominated by elevation, soil temperature, soil pH, disturbance and total N, our results are consistent with Malik, (2005) in which elevation was significantly important to establish plant communities. Elevation is among the most important factors affecting the vegetation distribution because it has direct impact in microclimate and living conditions of plant species (Singh *et al.*, 2009; Vittoz *et al.*, 2010). Some scientists also described that within the same altitude, soil properties were also determinant of vegetation composition (Amjad *et al.*, 2014a). Soil pH, soil temperature, Disturbance and Soil Phosphorus were significantly correlated to elevation which suggested that it was a key variable for affecting the communities of *G. macrophylla* (Larsen & Olsen, 2007). Soil nutrients were significantly correlated with total nitrogen, therefore, its variation could affect all other nutrients and ultimately affect the *G. macrophylla* communities. This result is in line with Fosaa, (2004) which indicated soil nutrients as most important factor for variation and distribution of plant communities in arid and semi-arid regions.

Species diversity measures the complexity of vegetation type and structure (Amjad *et al.*, 2014b). If species diversity varies in a study area, it refers to significant attributes of vegetation in that area (Brinkmann *et al.*, 2009). Spatial variation in species richness, heterogeneity and evenness was significant in eight communities. It corresponds to changes in structure and distribution of *G. macrophylla* communities and also had relation to environmental variables (Zhao *et al.*, 2004; Littell *et al.*, 2010). All diversity indices proved significant impact of elevation and soil temperature on species diversity. Species richness and heterogeneity decreased in the middle of elevation (1902-2102 m). Main reasons for low diversity in middle could be high tourists' interaction, over grazing, removal of plants for medicinal purposes and disappearance of annual plant species (Ram *et al.*, 2004). As previously, Zhang *et al.*, (2012) also indicated the impact of tourism and topography on vegetation diversity in the sub alpine meadows of Dongling Mountain of Beijing, China. Species diversity also varied with the variation of soil temperature because physio-chemical properties of soil also affect the phytodiversity (Larsen & Olsen, 2007).

Conclusion and Recommendations for *G. macrophylla* conservation: Environmental factors significantly affect the diversity and distribution of *G. macrophylla* communities. Interrelation and correlation studies of topographic and soil factors could be used for the management and conservation of *G. macrophylla* as it is highly uprooted for medicinal purposes and has very limited habitat in Northern China. It's uprooting and digging should be minimized, improved habitat conditions by fertilization and irrigation, and minimized disturbance to meadow by limiting the number of tourists and grazing for the sake of grassland rehabilitation. Cloning, cultivation and reproduction of this species are the best options for the scientists to meet the medicinal demand. Furthermore, monitoring of functional diversity of *G. macrophylla* communities is recommended because functional diversity can be used as an indicator to disturbance intensity and degraded communities.

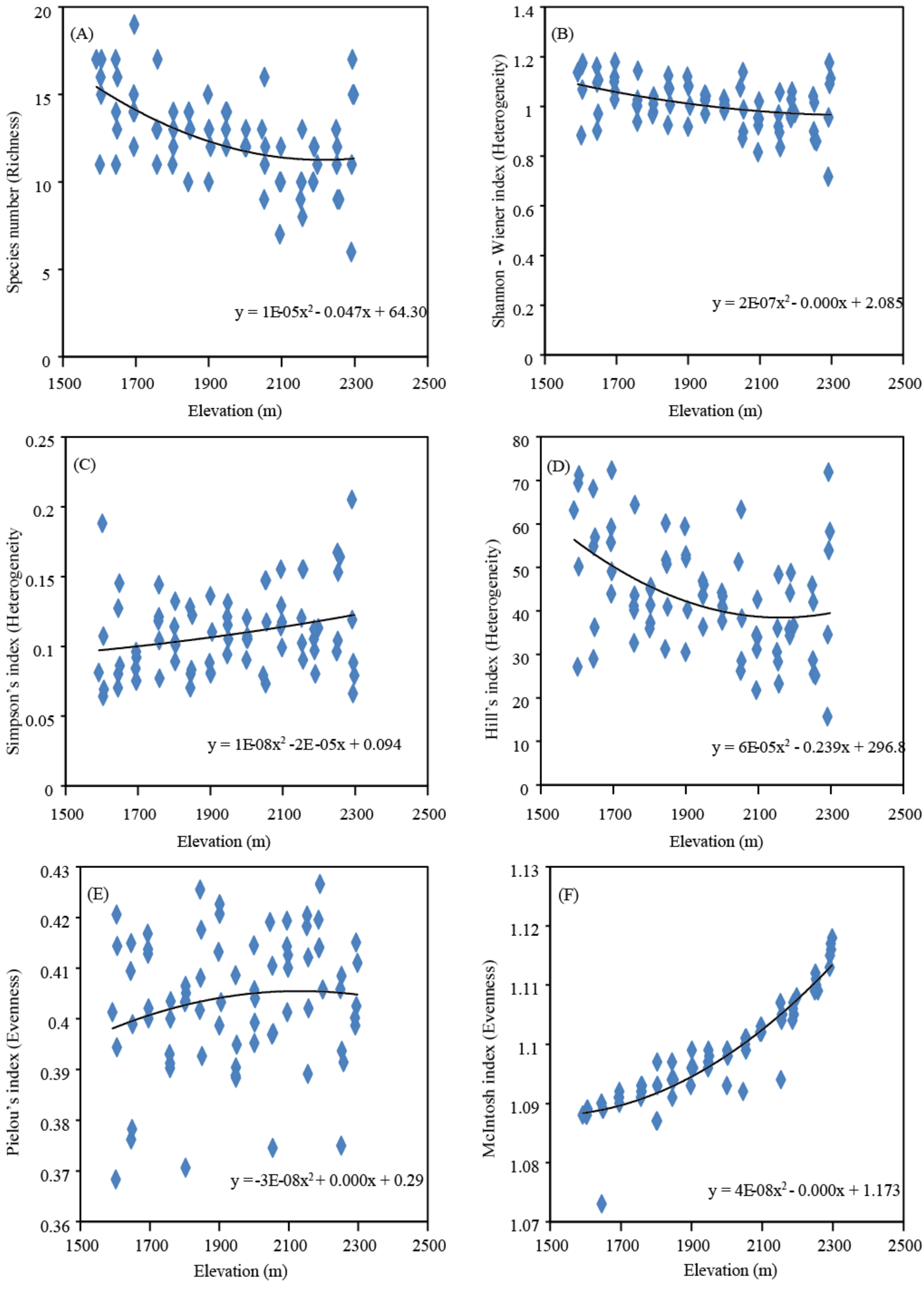

Fig. 3. Changes in species richness' heterogeneity and evenness of *G. macrophylla* communities along elevation gradient in Dongling mountain meadow, Beijing, China.

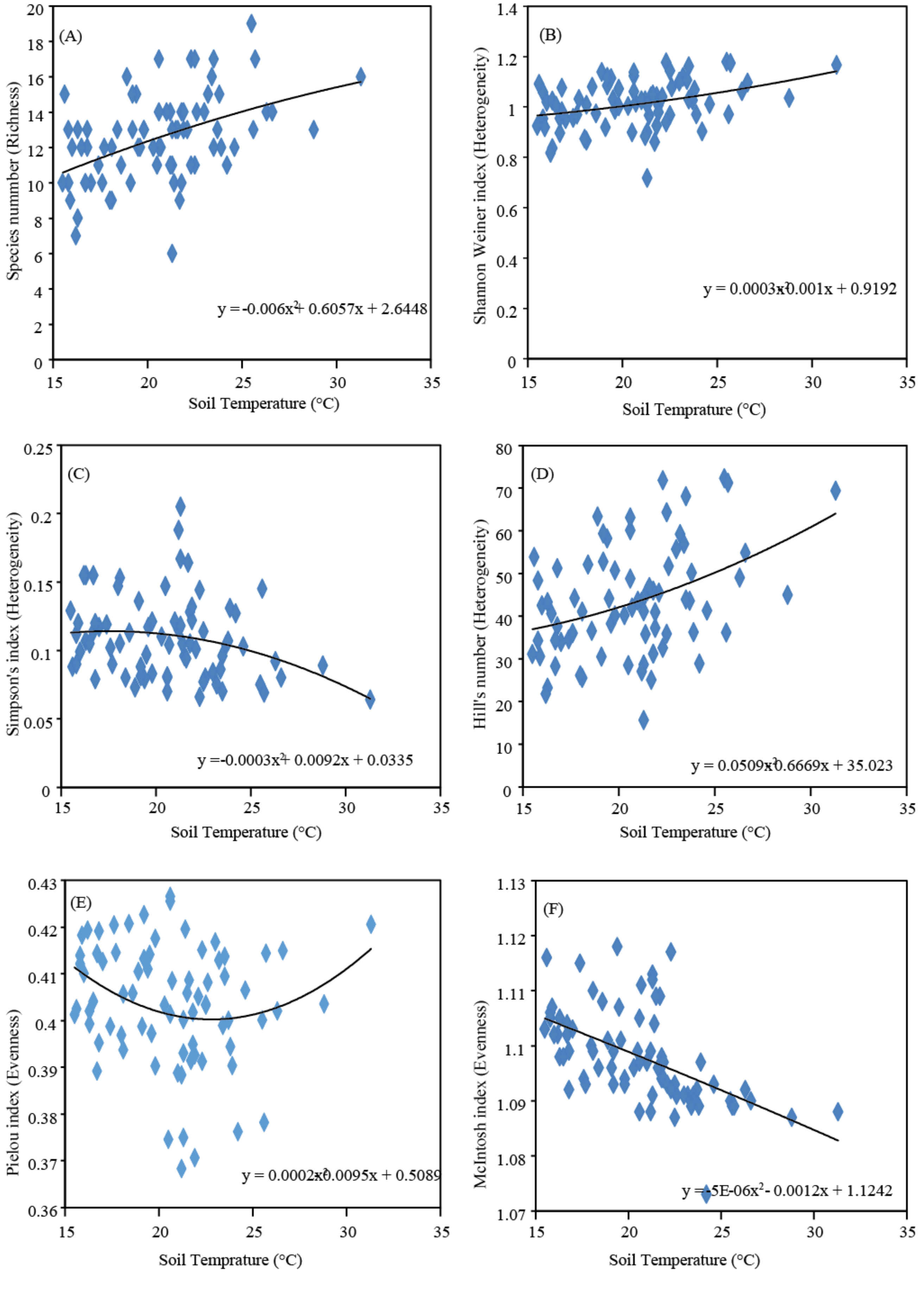

Fig. 4. Changes in species richness' heterogeneity and evenness of *G. macrophylla* communities with soil temperature in Dongling mountain meadow, Beijing, China.

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