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Yan-Bin JIANG [1], Xue-Hua LIU [2], Rui-Xuan TIAN [3], Xiao-Ming SHAO [1,*]

[1] College of Biological Sciences, China Agricultural University, No.2 Yuanmingyuan West Road, Haidian District, Beijing 100193, China
[*]e-mail: shaoxm@cau.edu.cn (*corresponding author*)
[2] Department of Environmental Science and Engineering, Tsinghua University Beijing 100084, China
[3] Shaanxi Guanyingshan Nature Reserve, Shaanxi 723400, China

FIELD-SAMPLING METHODS FOR INVESTIGATING GROUND-BRYOPHYTE POPULATIONS IN FOREST VEGETATION

ABSTRACT: To determine the optimum plot size for bryophyte-biocoenosis investigations and identify the sampling methods that can provide sufficient and representative data for bryophyte diversity, we explored two sampling methods and investigated the species composition and their coverage in three sites located within the subtropical-temperate zone in China: mixed coniferous and deciduous broadleaved forest with species of *Quercus*, *Betula, Pinus, Abies* (Guanyinshan Nature Reserve), mixed evergreen and deciduous broadleaved forest, broadleaved forest with species of *Phoebe, Castanopsis, Lithocarpus, Acer, Tilia, Dacidia, Abies* (Mabian Dafengding Nature Reserve) and temperate deciduous broadleaved forest with species of *Quercus, Betula, Populus* (Dongling Mountains). Two sampling methods are applied: the systematic-sampling method based on nested quadrates of five different sizes (10 × 10 cm, 25 × 25 cm, 50 × 50 cm, 1 × 1 m, and 2 × 2 m) in each 2 × 2 m grid within a 10 × 10 m plot (total 25 grids) and the microcoenose-sampling method (sampling with the minimum area quadrate at the center of the largest fragment in each of the 25 grids). The minimum area of sampling was determined based on the similarity-area curve, the coverage-area curve, and the importance-value-area curve through the systematic-sampling. The appropriate sampling method and quadrate number were determined by analyzing the species diversity and evenness. We compared two sampling methods by assessing the species number at two different sites. Both the similarity-area curve and importance-value-area curve showed that the turnover point of sampling size occurred at 50 × 50 cm where the similarity and importance-value were closer to the actual. We concluded that a quadrate of 50 × 50 cm could be used as the minimum area of sampling. However, the systematic-sampling method was not suitable for analyzing the diversity of bryophytes. A viable alternative is the microcoenose-sampling method which allows to obtain sufficient information in terms of species richness and their distribution.

KEY WORDS: bryophytes, systematic-sampling method, species diversity, minimum area, microcoenose-sampling method

1. INTRODUCTION

It is essential to develop a standardized approach for quantifying species abundance in different plant communities. A standardized sampling technique for measuring plant diversity is required to improve resource inventories, study the large-scale changes in abundance, and monitor long-term trends (Stohlgren *et al.* 1995, Raisa and Juha 2003).

In the species-diversity studies conducted at different spatial scales, such as local, regional, and landscape scales, the measure-

ment of the relationships, comparison of the results, and even predictions of species distribution can only be performed when a common methodology is employed for all the studies. Since the determination of the worldwide distribution and diversity of species and the identification of the factors influencing species distribution are important research objectives, a significant amount of attention is being paid to the identification of large-scale species-abundance patterns (Rahbek 2005).

While the modified Whittaker nested-vegetation sampling method (Shmida 1984) is a more or less standard sampling procedure for spermatophytes (Stohlgren *et al.* 1995), there has been no consensus on a standardized field methodology for evaluating the diversity of bryophytes and on the appropriate quadrate size to be used in such studies (Slack 1984, Podani *et al.* 1993). Some explaining could be the small size and the often fragmentary nature of bryophyte communities, the tendency to grow on highly irregular surfaces, their occurrence with relatively low biomass in communities dominated by other vegetations, and their high habitat heterogeneity influenced by microhabitat factors operating over short distances (Steel *et al.* 2004). However, there is a common agreement about the quadrate method which is used in almost all the studies on distribution and diversity (Table 1). Whereas, from these sampling approaches, some questions arise: (1) small size of quadrates may miss some species with low abundance or limited distribution; (2) community richness from different sites with different sampling schemes can not be compared effectively; and (3) how to evaluate larger-scale richness pattern?

The selection of the optimum plot size is influenced by various textural and structural properties of the community (Barkman

Table 1. Sampling methods used by different authors for analyzing ground bryophytes.

Author	Study site	Aim	Sampling method	Subplot size	Quadrat size	No of quadrates.
Ah-Peng *et al.* (2007)	a recent lava flow of the Piton de la Fournaise volcano	diversity and distribution	random	10 × 10 m	50 cm^2	15
Bai *et al.* (1998)	Helan mountain, China	diversity and biomass	random	–	10 × 10 m 1 × 1 m 10 × 10cm	Each 10–20
Cao *et al.* (2004)	garden and chemical factory in Shanghai city, China	distribution patterns	random	–	20 × 20 cm	10–16
Guo and Cao (1999)	main ecosystems in Changbai Mountain, China	diversity and distribution patterns	systematic	20 × 20 m	50 × 50 cm	72
Heino and Virtanen (2006)	Koutajoki drainage basin	distribution and abundance	random or systematic	100 m^2	50 × 50 cm	8 or 10
Jägerbrand *et al.* (2006)	alpine tundra in Latnjajaure, northern Sweden	diversity	according to dominant species	10 × 10 m	0.25 m^2	10–16
Li *et al.* (2006)	West Tianmu Mountain,China	diversity	systematic	10 × 10 m	50 × 50 cm	20
Økland *et al.* (2004)	understorey vegetation in Norway	vegetation-environment relationships	restricted random sampling	16 m^2	1 m^2	2
Pharo (2000)	a forest management district of eastern Australia	diversity	random	20 × 50 m	1 × 1 m	5
Xie (2003)	Jinhua city, China	distribution patterns	5 point' systematic	–	20 × 20 cm	5
Ye *et al.* (2004)	dark coniferous forest of Chan- gbai Mountain, China	bryophyte biomass	5 point' systematic	100 × 2 m	20 × 20 cm	50

1989). Therefore, we used the minimum area, which is often used to refer to a certain small region of the biocoenosis, containing a number of basic species and forming a 'coenotic molecule' that repeats more or less regularly throughout a certain environment. Since bryophyte communities are fragmentary and show high heterogeneity in their habitats, grassland bryophytes are generally believed to be poor competitors of vascular plants and bryophyte abundance declines as the vascular-plant coverage increases (van Tooren *et al*. 1988). Bryophytes are sensitive to acid deposition and the level of nutrients in the atmosphere (Bates 1992). Rahbek (2005) reaffirmed that the species-abundance patterns and mechanisms were scale invariant and illustrated these results by using examples and a quantitative analysis of the published data. There are several approaches for determining the minimum area for vascular plants: nested-quadrate (Mueller-Dombois and Ellenberg 1974), long-thin plot design (Stohlgren 1994), and modified Whittaker plot (Stohlgren *et al*. 1995). All these approaches were based on the quantification of species-area curves, which is the original and frequently recommended method. However, the number of species rarely reaches a saturation level (Peet 1974). The similarity analysis is another approach for minimum-area determination and was originally carried out by analyzing series of randomly located plots with increasing size and determining the point where the mean pairwise floristic similarity between sample plots exceeded 80%. While analyzing a phytocoenosis, the minimal area is reached as the basic set of species of the phytocoenosis is present in all the sample plots (Moravec 1973). It also can extend the similarity calculations to analyze the quantitative data, to create a dominance index in which higher similarity levels are indicated in terms of the species dominance in series of nested plots with increasing size (Dietvorst *et al*. 1982).

This study was conducted to fulfill the following objectives: (1) to determine the minimum area for investigating ground bryophytes, and (2) to discuss an appropriate sampling method that can be used to compare different vegetations from different areas with various spatial scales by using the same standard.

2. MATERIALS AND METHODS

2.1. Study area

The study was conducted in three sites (Fig.1, Table 2). Guanyinshan Nature Reserve (GYNR) is located in the central region of the southern slope of the Qinling Mountains, which represents the ecotone between the warm-temperate and subtropical zones in China and plays the important role of a natural geographical barrier that impedes cold airflow from the north and forms the northernmost refuge of the Giant Panda. Its elevation ranges from 1150–2574 m. There is a very rich population of plants and the natural vegetation shows an obvious vertical distribution spectrum from deciduous broadleaved forest to coniferous forest. The constructive species are *Quercus, Betula, Pinus and, Abies* (Dang *et al*. 2006). Mabian Dafengding Nature Reserve (MDNR) is located at the east slope of the Daliangshan Mountains which is the windward slope at the south-east monsoon, and has a subtropical humid climate. Its flora belongs to Central China of China-Japan forest vegetation subregion. It has a lowest elevation of 800 m and a highest elevation of 4042 m. From the mountain bottom to the top, it is covered by the evergreen broadleaved forest, mixed evergreen and deciduous broadleaved forest, mixed broadleaved and coniferous forest, dark coniferous forest, sub-alpine shrubs, and meadow. The dominating tree species are *Phoebe, Castanopsis, Lithocarpus, Acer, Tilia, Dacidia, Abies* (Luo 2003). Dongling Moun-

Table 2. Characteristics of three study sites.

Study sites	Acronyms	Coordinates	Main forest species
Guanyinshan Nature Reserve	GYNR	33°35'–33°45'N 107°51'–108°01'E	*Quercus variabilis, Betula albo-sinensis, Pinus armandii, Abies chensiensis*
Mabian Dafengding Nature Reserve	MDNR	28°26'–28°47'N 103°13'–103°25'E	*Abies fabri, Davidia involucrata, Lithocarpus megalophyllus + Acer spp., Castanopsis fargesii*
Dongling Mountain	DLM	40°00'–40°02'N 115°26'–115°30'E	*Quercus liaotungensis, Betula platyphylla, Populus cathayana, Pinus tabulaeformis*

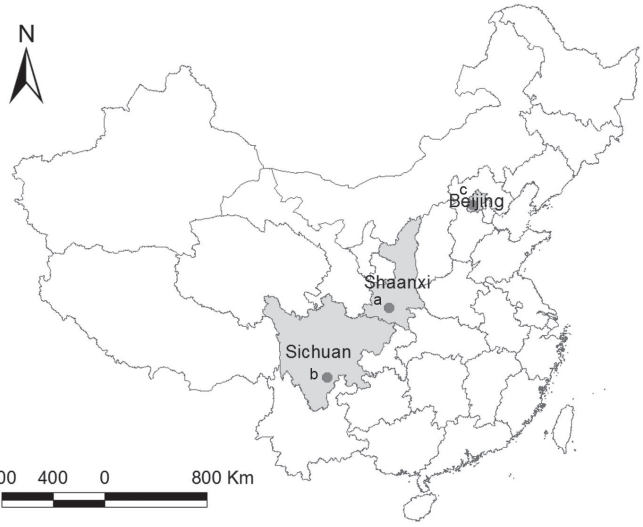

Fig. 1. Location of the study sites (shown in grey) on the map of China, a – GYNR (Guanyinshan Nature Reserve), b – MDNR (Mabian Dafengding Nature Reserve), c – DLM (Dongling Mountain).

tains (DLM) is located at Qingshui Town of Mentougou District in the west of Beijing. Its maximum elevation is 2303 m. It has a continental warm temperate monsoon climate. Its zonal vegetation belongs to warm temperate deciduous broadleaved forest and the dominating species are *Quercus, Betula, Populus* (Lou 2004).

2.2. Sampling Methods

2.2.1. Systematic-sampling method and minimum area of sampling

We applied the systematic-sampling method to finding out the minimum area of sampling. Total 15 plots all with same size of 10 × 10 m were selected in the forest with five in each site (Fig. 1). Each plot was delineated to 25 grids with same sizes of 2 × 2 m. The nested quadrates were located systematically shown in Figure 2 and the distance between the starting quadrates of adjacent series were kept constant. In each of the 25 grids, five successive enlarging nested quadrates, with dimensions of 10 × 10 cm, 25 × 25 cm, 50 × 50 cm, 1 × 1 m, and 2 × 2 m, were set up to investigate the coverage of all the ground-bryophyte species.

We defined the number of species obtained from all nested quadrates investigation within all 2 × 2 m quadrates (*i.e.* total sampling) as the actual number of species. Coverage of bryophytes was measured using a metallic quadrate divided into 100 grids. We then counted the grids number that bryophytes covered. The specimens were collected from the sites, air dried and identified to the species level in the laboratory. After data analysis, we can obtain the minimum area of sampling.

2.2.2. Microcoenose-sampling method

In the microcoenose-sampling method, we considered each bryophyte fragment as a microcoenose. If there were several fragments, we chose the largest fragment and marked the minimum area quadrate obtained from section 2.2.1 at the center of each of the 25 grids and measured its species presence and coverage. We also noted if there was no bryophyte in one of the quadrates.

Data collection was performed in GYNR in July 2007 and 2008, and in MDNR in August 2008. Data of DLM was obtained from the paper published by Sun *et al.* (2007). All the specimens were stored in China Agricultural University herbarium.

2.3. Data analysis

We used the following indices in this study (Sprensen 1948, Whittaker 1972, Pielou 1975, Wang *et al.* 1996, Zhang 2004):

Patrick index of species richness:

$$C = S \quad (1)$$

where S is the number of species in a plot, here with 10 × 10 m size.

Similarity index of species composition:

$$S_s = 2c / (a + b) \quad (2)$$

where a, b and c are respectively the total number of species in the first sample, the total number of species in the second sample, and the number of species common to both samples.

Importance value:

$$D = (Ni + Fi) / 2 \quad (3)$$

where Ni is the relative coverage of 'i' species, and Fi is the relative frequency.

Shannon-Wiener index of diversity:

$$H' = -\sum_{i=1}^{s} P_i \ln P_i \quad (4)$$

where $Pi = Ni/N$, in which Ni is the relative coverage of 'i' species, and N is the summation of the coverage of all S species. H' reflects the species diversity.

Pielous index of eveness:

$$E = H'/\ln S \quad (5)$$

E reflects the evenness of species.

Varp index of species-distribution pattern:

$$S^2 = \frac{\sum f(x - \bar{x})^2}{N' - 1} \quad (6)$$

where S^2 is the sample variance and N' is the number of nested quadrates, here, we considered N' to be 25, $N' - 1$, degree of freedom (df), and x, the species number in each nested quadrate. $S^2/\bar{x} = 1$ indicates the Poisson's distribution, $S^2/\bar{x} > 1$ indicates the contagious distribution, and $S^2/\bar{x} < 1$ indicates the even distribution.

We chose 35 nested quadrates to investigate the species presence-absence data and the coverage after excluding those grids and also nested quadrates without bryophytes. The species similarities for all-sizes nested quadrates (*i.e.*, 10 × 10 cm, 25 × 25 cm, 50 × 50 cm, 1 × 1 m, and 2 × 2 m) to the 2 × 2 m quadrates were calculated by using formula (2) to obtain the average values for each size. The similarity-area curve was used to determine the qualitative minimum area. Then,

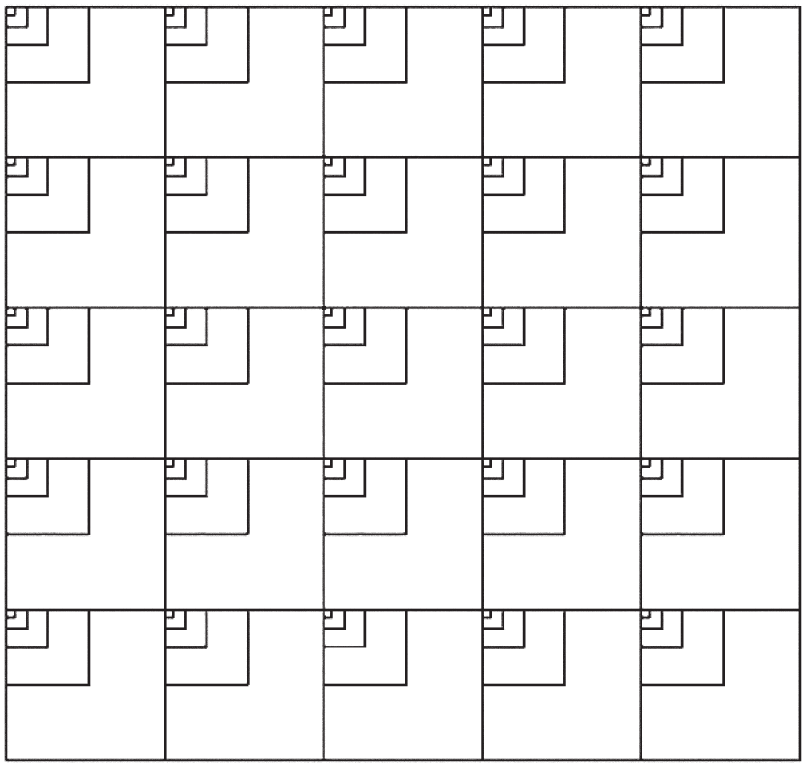

Fig. 2. Arrangement of the various-size quadrates in the standard minimum area determination. The total area of the plot has a size of 10 × 10 m and was delineated to 25 grids with size of 2 × 2 m. Each grid includes 5 nested quadrates with sizes of 10 × 10 cm, 25 × 25 cm, 50 × 50 cm, 1 × 1 m, and 2 × 2 m.

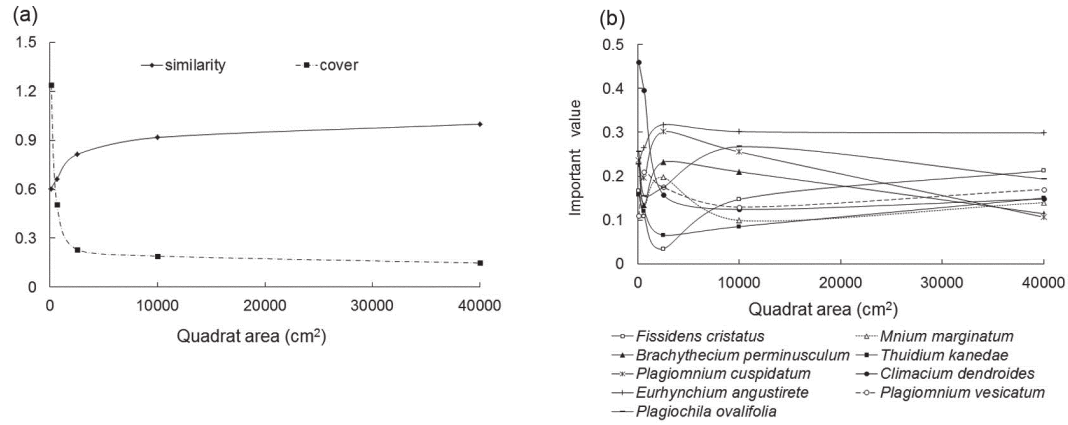

Fig. 3. Similarity-area and coverage-area curves; (a) an importance-value-area curve; (b) when the curve reached the turnover point, we considered the corresponding area as the minimum area. On the basis of the above results, we determined 50 × 50 cm as an approximate minimum area.

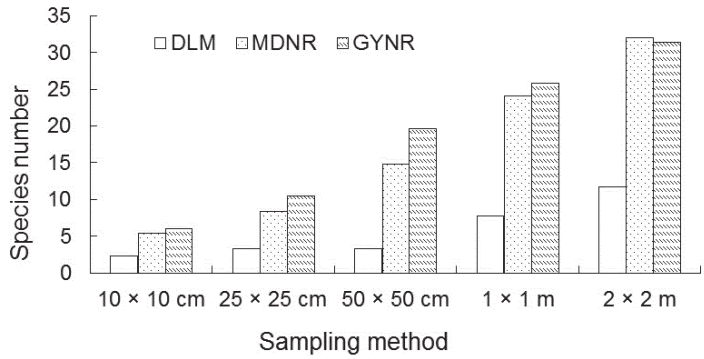

Fig. 4. Comparison of average number of species for 10 × 10 m plots in three different sites using the same systematic-sampling method. DLM, MDNR and GYNR – for site acronyms see Fig. 1 and Table 2.

the quantitative aspect was determined by measuring the bryophytes coverage in enlarging nested quadrates. The importance-value-area curve of dominant species in these quadrates was used to verify the minimum area.

The appropriate sampling method and quadrate number were determined by using the Shannon-Wiener index and Pielous index. We analyzed species diversity, evenness, and relationships between different sampling methods. The species-distribution pattern can be determined from the ratio of S^2 and $\overline{x}$. We also compared the measured species number in microcoenose-sampling method with the actual species number in each plot at two different sites. Two tailed T-test was used to test their difference, and it was thought to be no significant difference when $P > 0.05$.

The statistical analyses were performed using SPASS 14.0 and BIO-DAP software.

3. RESULTS

3.1. Minimum area determination based on the systematic-sampling method and its inaccuracy

Minimum area determination was the first step for developing an appropriate sampling method to study bryophytes. We tested both qualitative and quantitative relationships of the quadrate areas, and Fig. 3 shows the respective relationships. This figure shows that the similarity values at a quadrate size of 50 × 50 cm were already greater than 80%, and there were no significant differences in the curve characteristics for the subsequent quadrate sizes. However, there were no significant changes in the coverage-area curves from the quadrates 50 × 50 cm to 2 × 2 m. The turnover point of the curve was deter-

Table 3. Detection of species-distribution patterns for various quadrate sizes. $S^2/\bar{x} = 1$ indicates the Poisson's distribution, $S^2/\bar{x} > 1$ indicates the contagious distribution, and $S^2/\bar{x} < 1$ indicates the even distribution. Site/plot acronyms – see Table 2.

Sample plot	$S^2/\bar{x}$				
	10 × 10 cm	25 × 25 cm	50 × 50 cm	1 × 1 m	2 × 2 m
GYNR01	0.800	0.720	2.847	1.952	1.77
GYNR02	1.000	1.040	1.067	1.015	1.006
GYNR03	0.720	0.667	1.037	1.262	1.420
GYNR04	0.560	0.502	1.122	0.985	1.276
GYNR05	1.192	0.735	0.619	0.783	0.949

mined on the basis of the following assumption: the sampling area can be the minimum area when the area increases by 10% and the total species number increases correspondingly only 10%. The importance-value-area curve showed that the turnover point occurred at sampling size of 50 × 50 cm, where the dominant species' important-values in the communities were closer to the actual values. On the basis of these results, we selected 50 × 50 cm as an approximate minimum area for sampling.

The systematic-sampling method has been used in a number of studies on bryophytes, which was adopted in this study. The data obtained from three different sites showed that the values for the total species number in the 25 quadrates for 10 × 10 m plot at all size levels were not equal to the corresponding actual species number (Fig. 4), which meant that systematic-sampling using a 50 × 50 cm quadrate could lead to inaccuracy in the determination of the number of species. In GYNR, for the 50 × 50 cm quadrate, there were in average only 20 species in comparison with 31 species identified in the 2 × 2 m quadrate. We also obtained similar results for the other two locations: MDNR and DLM, for which the corresponding values for the 50 × 50 cm quadrates and the 2 × 2 m quadrates were in average 15 and 32, and 3 and 12, respectively. Enlargement in the quadrate size corresponded to a more or less linear increase in the species number. Therefore, the results obtained by using this sampling method for species diversity would be inaccurate. Moreover, it was obvious that the number of species in DLM was much less than that in the other two sites, this discrepancy can be attributed to the effects of environmental factors, latitude, precipitation, and the secondary forest vegetation (Sun *et al.* 2007).

The Shannon-Wiener index (*i.e.*, diversity index) and Pielous index (*i.e.*, evenness index) revealed that species diversities in five plots in GYNR were similar, but the evenness of species of all the plots was rather low, ranging from 0.53 to 0.72 (Fig. 5). This result showed that the species in this area are distributed heterogeneously, and they may cluster or disperse on the ground in the form of numerous patches (Table 3). Since the information obtained from quadrates smaller than 50 × 50 cm is inadequate, the $S^2/\bar{x}$ values for these quadrates can be ignored, while the information for the other quadrates (50 × 50 cm and larger) generally showed contagious distribution. Therefore, the systematic-sampling method using smaller quadrates may lead to the omission of important patches of bryophytes and under-representation of species richness in the plot.

3.2. The microcoenose-sampling method with considering the minimum area of sampling

Then, we used another sampling method, *i.e.*, the microcoenose-sampling method with considering the minimum area of sampling for GYNR. In this method, the minimum area of sampling (50 × 50 cm) quadrate was selected at the center of microcoenoses in each of the 25 grids, and the species presence-absence data and coverage were obtained. The most significant difference between this method and the systematic-sampling method can be seen from the number of species recorded in same minimum areas in all 25 quadrates in the 10 × 10 m plot (Table 4 and

Table 4. Comparison of the species number measured by using the microcoenose-sampling method with the actual species number in each plot at different sites: GYNR (Guanyinshan Nature Reserve) and MDNR (Mabian Dafengding Nature Reserve).

Sample plot	Measured species number [a]	Actual species number [b]	Quadrate number with bryophytes [c]	Two-tailed T-test for measured species number and actual species number
GYNR01	31	33	23	
GYNR02	34	34	24	
GYNR03	30	31	20	$P = 0.558$ no significance
GYNR04	29	29	25	
GYNR05	29	30	24	
GYNR-average	31	31		
MDNR01	25	25	17	
MDNR02	29	29	20	
MDNR03	34	35	22	$P = 0.851$ no significance
MDNR04	28	28	20	
MDNR05	40	43	25	
MDNR-average	31	32		

Note: [a] – species number from the microcoenose-sampling method based on 50 × 50 cm (minimum area of sampling); [b] – species number from all 2 × 2 m quadrates using total sampling; [c] - the number of quadrates in which bryophytes occurred within 25 grids in each 10 × 10 m plot.

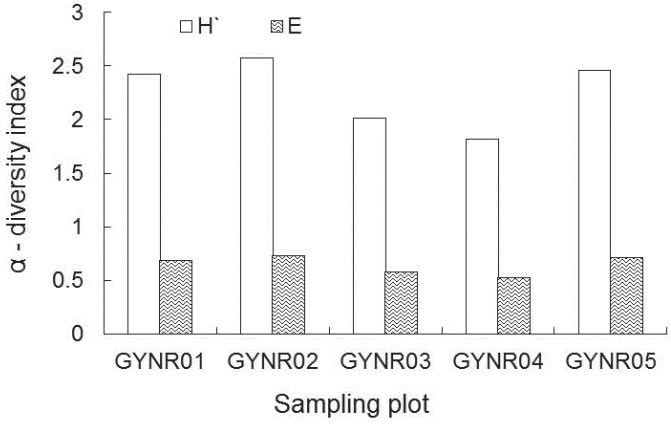

Fig. 5. Species diversity in five sample plots in Guanyinshan Nature Reserve (GYNR). $H^{`}$ is the Shannon-Wiener index to show species diversity, and E is the Pielous index to show evenness of species.

Fig. 6). Using this microcoenose-sampling method, the corresponding number of species based on minimum area of sampling was 31 for 10 × 10 m plot, while the systematic-sampling method only obtained 20 species for 10 × 10 m plot under the same minimum area of sampling. As described previously, the total sampling with all 2 × 2 m quadrates showed 31 species for plot. Thus, the species number derived from the systematic-sampling method was approximately 65% of the actual species number in the plot, while our improved microcoenose-sampling method obtained 100% species.

To test the appropriateness of this method, we used it at another site, MDNR, and the results were shown in Table 4. These results showed that there was no significant discrepancy ($P > 0.05$) between the species abundance calculated by microcoenose-sampling and the actual species number determined in total sampling. Bryophytes distributed more discontinuously in MDNR, often in less than 25 quadrates. The lowest number of quadrate

with species was 17. These results showed that the improved microcoenose-sampling method is more effective when bryophytes are absent in some areas.

4. DISCUSSION

4.1. Comparison with species-area determination

To determine the minimum area, we primarily used the species-area curve, which is a measure of the species abundance in an area. The species-area curve in our study did not reach saturation (refer to the species-area curve for bryophytes with the same data in the 5 sample plots in GYNR).

Why do bryophytes cluster or disperse as patches? Laaka-Lindberg *et al.* (2003) considered that asexual propagules play a significant role in bryophyte dynamics. While spores are the sexual means of reproduction and dispersal of mosses, providing a mechanism for recombination and variation, it is likely that most mosses show more reliance on various vegetative methods for their propagation (Anderson 1963, Steere 1965, Longton 1976, 1982, Selkirk 1984). In the case of the ground species, birds and other animals scratching among the plants dislodge numerous fragments (Selkirk 1984). These fragments, which are capable of regenerating into new plants, are dispersed by winds. Water may also aid in the dispersal of bryophytes in various ways. Bryophytes adhere to soil with their rhizoids, and when they are struck by raindrops, they may easily float away. Moreover, the fertilization of bryophytes also depends on water. These all may be the factors. However, bryophyte patches are not homogeneous, since they occur as conjunct or mixed groups. Other factors such as temperature, humidity, light, and coverage of vascular plants may have an impact on the distribution pattern (Pharo *et al.* 2000, Mills and Macdonald 2005), and some species may grow only in specified habitats. Consequently, even in a limited sample plot with microhabitat variance, some species may be present while some others may be absent. As a whole, the species number increases with an enlargement in quadrate size if there are any changes in the environmental factors that influence bryophytes.

4.2. Application of the minimum area of sampling (*i.e.* 50 × 50 cm quadrate) and microcoenose-sampling method

The assessments for different kinds of vegetation may require different standards. Cain and Castro (1959) advised that the investigations for northern-temperate-zone bryophytes should be performed with sample quadrates of 0.01–0.1 m^2. However, the investigations for epiphytic bryophytes should

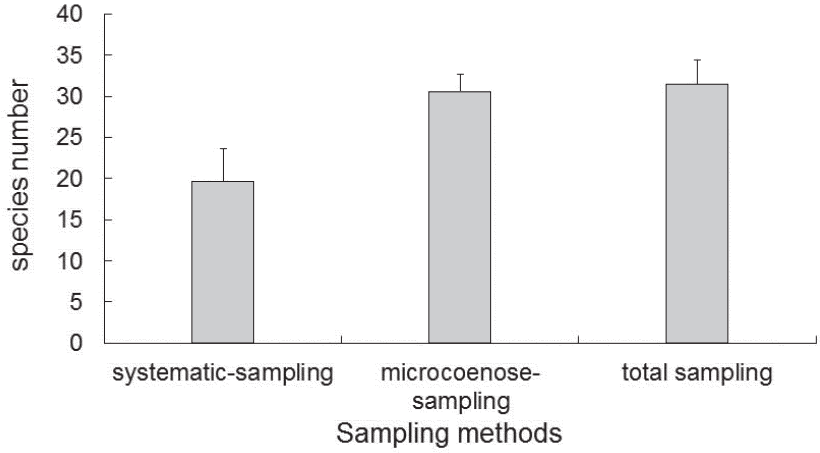

Fig. 6. Average species number in the 10 × 10 m plots in Guanyinshan Nature Reserve (GYNR), the number of species was obtained by using both systematic-sampling and microcoenose-sampling methods within 25 quadrates of 50 × 50 cm (minimum area of sampling) and 2 × 2 m (total sampling) respectively.

be performed with much smaller quadrates, since the areas of communities on tree trunks are rarely greater than 0.04 m^2 (Iwatsuki 1960). While this minimum area is used for assessing ground bryophytes, especially those growing on flat ground, the abundance calculations for other habitats such as tree trunk, rotten wood, and sloped stone have still not been clarified. For the assessment of tree-trunk bryophytes, a quadrate size of 50 × 50 cm is too large, because the diameter of breast height (DBH) is not compatible with this size, and the properties of the species growing on tree trunks show a significant correlation with tree height. Consequently, the factors influencing bryophyte distribution may change within the same quadrate.

For meadow, tundra, and peat-lands, which show more homogeneous vegetation, the minimum area of sampling (*i.e.*, 50 × 50 cm quadrate) obtained from this study is expected to be sufficient for investigating both diversity and distribution of species. Without considering time and personnel consuming, a systematic-sampling method can be adapted to some extent, sampling at random also can be accepted, but less information obtained compared with microcoenose-sampling.

Using the microcoenose-sampling method, we can obtain sufficient information in terms of species richness and distribution. However, when we investigate according to the microcoenose, there will be some quantitative data needed to deal with, such as abundance, biomass, *etc*. Coverage measuring is the current method for quantitative analysis used in this study. It is essential to identify a method to transform the data obtained from the quadrates to represent the plot or vegetation.

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