

Spatial variation in community structure of a subtropical evergreen broad-leaved forest: Implications for sampling design

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With the full survey data for a 24-ha subtropical evergreen broad-leaved forest dynamics plot, we evaluated spatial variation in forest structure characteristics (basal area and aboveground biomass), and calculated the minimal sample size and total sampling area necessary to estimate the forest structure characteristics within 20% ($\pm 10\%$) of the observed values with 95% probability for particular quadrat sizes by using a computer program that is designed to simulate the sampling process by allowing different sized quadrats to be randomly located within the sampling region. We found that (1) based on the 600 20 m \times 20 m subplots, basal area and aboveground biomass displayed a high degree of variation, with respective coefficients of variation of 27% and 31%; (2) based on the computer simulation analysis, the variability of basal area and aboveground biomass decreased with increasing quadrat size. The number of quadrats required to achieve the specified degree of precision dropped sharply with the increase of quadrat size. However, the total sampling area increased with increasing quadrat size, suggesting that using several small quadrats across the sampling area is more efficient than using fewer larger quadrats. Results of this study are valuable for evaluating the reliability of previous research and may assist researchers in designing effective sampling strategies for future field surveys, particularly in subtropical evergreen broad-leaved forests in China.

spatial variation, basal area, aboveground biomass, quadrat size, sample size

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As one of the most important ecosystems on the earth, forest not only provides human beings with food, medicine and many kinds of raw materials, but also plays an important role in climate regulation, water conservation and environment maintenance, etc. [1]. Just because of its vital role in the development of human society, forest gains great concern worldwide and becomes an important research object of science. With the intensification of species loss, global climate change and other environmental issues, forest species coexistence mechanism and the role of forest ecosystems in global carbon cycles become the central topic of current ecological studies [2,3]. In recent years, ecologists have presented many scientific hypothesis based on extensive observational and experimental studies. For example, it was found through long-term monitoring that the biomass of

old-growth tropical forest had significantly risen in the past few decades [4,5]. Therefore, some scholars proposed a hypothesis that primary forests or old-growth forests might still be carbon sinks [5], which challenged the traditional view that the community structure and functions of mature forest tend to dynamic equilibrium [6]. Due to the lack of historical data on forest disturbance, these conclusions have caused bigger controversy [7]. As a result, the future development trend is to carry out further studies to verify the hypothesis or propose new hypotheses.

Sampling survey is an important approach for forest ecological studies, and lots of experience and theories are obtained and verified based on the analysis of quadrat survey data [8]. Due to the influence of environment, community process, and natural or human disturbance, the community structure tends to have strong spatial variability. For example, influenced by the topography, soil nutrient, light

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condition, disturbance and other factors, the spatial distribution of the aboveground biomass at Barro Colorado Island, Panama showed significant variability [9,10]. Spatial variability of community structure brings large difficulties to community survey. Whether community survey can accurately reflect the true character of the community structure depends on whether the chosen sampling area and the number of quadrats are representative. In actual survey, people tend to choose well-grown or undisturbed forest as the survey object, which may cause a substantial deviation of results [11]. Ideally, the larger the sampling area is and/or the more the number of quadrats is, the more accurately the true character of community can be reflected. However, subject to constraints on funds and survey time, the ideal state cannot be achieved. Therefore, it is necessary to design appropriate quadrat size and sample size in order to obtain the most representative community survey data on the basis of minimum funds and time [12]. As to sampling design for community survey, many studies have been carried out in tropical and temperate forests [8,9,13–17]. However, although the subtropical evergreen broad-leaved forests are widely distributed in China [18], and the domestic scholars have conducted lots of studies on the community structure of subtropical evergreen broad-leaved forests in the past several decades (Table S1), few of them touched upon the sampling design based on comprehensive survey data. Most previous studies were based on a fixed small plot or small number of quadrats (Table S1). For example, Yang et al. [19] estimated that the biomass of a 52-years-old *Schima superba*-*Castanopsis carlesii* forest in Tiantongshan, Zhejiang Province was 225.3 ± 30.1 Mg/ha based on 5 quadrats with a size of 20 m × 20 m. How about its reliability? Whether or not the survey results based on the 5 quadrats sized 20 m × 20 m could truly reflect the community biomass? So far, there has not been a comparatively reliable evaluation basis.

The Gutianshan 24-ha forest dynamic plot of typical subtropical evergreen broad-leaved forest was established in 2005 [20]. All trees ≥ 1 cm in diameter at breast height were tagged, mapped, measured and identified to species [20]. Taking the basal area and aboveground biomass as the study objects, this study randomly samples different sized quadrats by computer simulation, and analyzes the spatial variability of basal area and aboveground biomass. We addressed two specific issues: (1) Assessing the spatial variability of community structure (basal area and aboveground biomass); (2) Calculating the minimum number of quadrats required for estimations of basal area and aboveground biomass to meet a specified accuracy when random sampling with different sized quadrats.

1 Methods

1.1 Study site and data collection

This study was carried out in Gutianshan National Nature

Reserve (GNNR) located in Kaihua County, Zhejiang Province, China ($29^{\circ}10'19.4''$ – $29^{\circ}17'41.4''$ N, $118^{\circ}03'49.7''$ – $118^{\circ}11'12.2''$ E). Total area of GNNR is about 8,000 ha. This place is characterized by subtropical monsoon climate, with a mean annual temperature of 15.3°C and a mean annual precipitation of 1964 mm [21]. The dominant soil can be classified into red, red-yellow, yellow-red and marsh soils, and soil pH ranges from 5.5–6.5 [21]. A total of 1426 seed-plant species of 648 genera and 149 families have been recorded as occurring naturally in GNNR [22]. The predominant vegetation type of GNNR is subtropical evergreen broad-leaved forest dominated by *Castanopsis eyrei*, *Schima superba* and *Cyclobalanopsis glauca* [21].

In 2005, a 24-ha permanent plot was established in the core area of GNNR (Figure 1) as part of the Chinese Forest Biodiversity Monitoring Network. The plot was divided into 600 subplots (20 m × 20 m) marked with 651 cement pegs. All trees with diameter at breast height (dbh) ≥ 1 cm were measured, tagged, mapped, and identified to species. We encountered 159 species belonging to 103 genera and 49 families in the plot [20].

1.2 Study objects selection

We selected the basal area and aboveground biomass as the objects of simulated sampling. Basal area is the cross-sectional area at breast height (1.3 m), and aboveground biomass is estimated using published allometric equations. The 11 species-specific and 1 genera-specific allometric equations were available for the common species of our study plot (Table S2). Where multiple equations were available for the same species, we used the one developed for the closest geographic location [23]. For those species that did not have species-specific or genera-specific allometric equation, the aboveground biomass was calculated by using the generalized equation of Zhang et al. [24] for subtropical forests in Zhejiang Province. Tree height was estimated via 47 species-specific and one generalized regression models developed from a sample of 1066 trees collected in our study plot (Table S3).

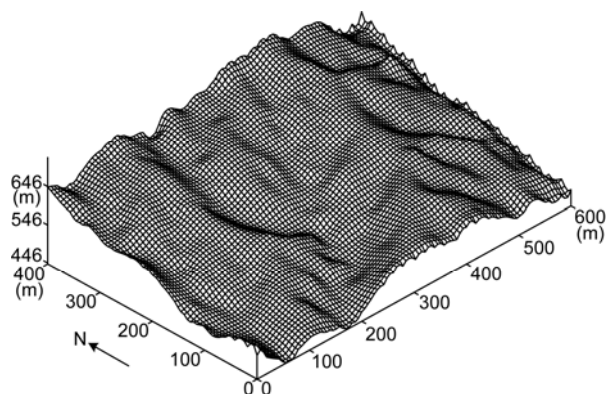


Figure 1 Topography of the 24-ha Gutianshan plot.

1.3 Simulated sampling

To ensure the simulation results include all possible variations, we use the following sampling method: Randomly select a point in the 24-ha plot and expand outward to specific side length (quadrat) centered on this point. Then calculate the basal area and aboveground biomass of each tree in the quadrat, and add together to calculate the total basal area and total aboveground biomass of the quadrat respectively. Finally, convert these values into the corresponding values per unit area. Repeat the steps listed above 10000 times for different sized quadrats. Side lengths of quadrats for simulated sampling were set as 10, 20, 30, 40, 50, 80, 100, 150 and 200 m, i.e. the minimum quadrat area is 0.01 ha and the maximum quadrat area is 4 ha. During simulated sampling, a buffer was supposed to be set at the edge of 24-ha plot according to the side length of quadrat to prevent quadrats from dropping out of the study plot. For instance, when the side length of quadrat is 10 m, the buffer width was set as 5 m.

1.4 Data analysis

The observed value of basal area and aboveground biomass were obtained through dividing the total basal area and aboveground biomass of the entire plot by the total area of the study plot (24 ha). We divided the 24-ha plot into 600 20 m×20 m subplot to assess the spatial variability of basal area and aboveground biomass, and used coefficients of variation (CV) to represent them.

Basic statistics (mean, standard deviation, maximum, minimum and CV) were calculated for all sampling distributions for every sized quadrat produced by random sampling as described above. The relationship between CV and quadrat area was analyzed using simple linear regression.

Based on the data set obtained through the 10000 times random sampling, we calculated the minimum number of quadrats required to ensure there was 95% probability for the estimated values to within 20% (±10%) of the observed values. The formula is as follows [8]:

$$n = \left[\frac{st_{0.05}}{d} \right]^2, \tag{1}$$

where n is the minimum number of quadrats; s is standard deviation of the sampling distributions; t is the Student's t -statistic (set to 1.96, without regard to its changes); d is the observed value ×10%. Besides, we calculated the error range where the estimated value had 95% probability to fall when randomly sampling 2, 4 and 6 quadrats sized 20 m×20 m and 30 m×30 m respectively. Simulated sampling procedures and subsequent analysis were performed using the R project software version 2.12.1 [26]. Simulated sampling routine can be found in Supporting Information (Text S1).

2 Result

2.1 Spatial variation of basal area and aboveground biomass

As shown in Figure 2, based on the 600 20 m × 20 m subplots, the basal area and aboveground biomass showed significant spatial variability. The maximum of basal area and aboveground biomass are 5.9 times and 6.5 times of the minimum respectively. The variability of aboveground biomass is greater than that of the basal area (Table 1).

Results of simulated sampling also showed that the basal area and aboveground biomass have significant variability (Figure 3). With the increase of quadrat area, their variability decreases gradually (Figure 3). The negative correlation between CV and quadrat area also indicates that their variability is relatively low when large quadrats are used for sampling (Figure 4). In this study, when the quadrat size is set as 10 m×10 m, their variability is maximum, and the CV of basal area and aboveground biomass is 47.2% and 52.7% respectively. Although sampling with comparatively larger quadrats significantly decrease the variability (Figure 3), their variability still cannot be ignored. For example, when the quadrat area is set as 1 ha, the estimated value of basal

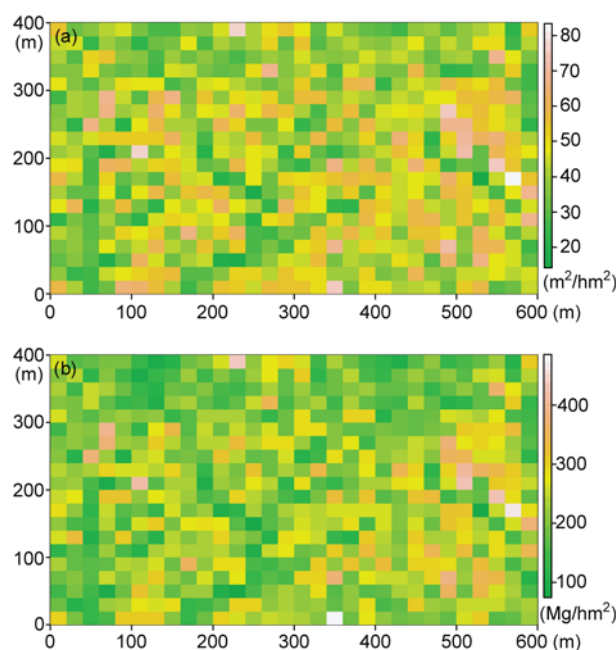


Figure 2 Spatial distribution of basal area (a) and aboveground biomass (b) based on the 600 20 m×20 m subplots.

Table 1 Variability of basal area and aboveground biomass based on the 600 20 m × 20 m subplots

Study object	Mean	Maximum	Minimum	CV (%)
Basal area (m ² /ha)	43.47	83.38	14.25	27
Aboveground biomass (Mg/ha)	223.02	490.69	75.60	31

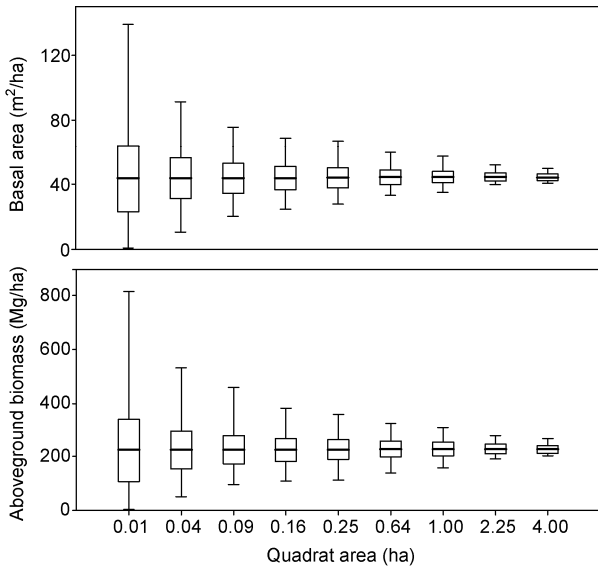


Figure 3 Variability of basal area and aboveground biomass at different quadrat area based on 10000 times random sampling. Broad line indicates the mean value; the box means \pm standard deviation and whiskers mean minimum and maximum values.

area range from 34.8 to 57.1 m²/ha, and the aboveground biomass range from 156.3 to 307.8 Mg/ha. Therefore, a fixed survey quadrat of 1 ha cannot accurately represent the true character of the surveyed community all the time.

2.2 Estimate of the minimum sample size

According to formula (1), we estimated the minimum number of quadrats required to ensure there was 95% probability for

the estimated values to within 20% ($\pm 10\%$) of the observed values (Table 2), and meanwhile calculated the error range where the estimated value had 95% probability to fall when randomly sampling 2, 4 and 6 quadrats sized 20 m \times 20 m and 30 m \times 30 m (Table 3). As shown in Table 2, with continuous increase in the quadrat area, the number of quadrats required decreases gradually while the necessary total sampling area tends to increase. For example, when sampling with quadrats sized 10 m \times 10 m to estimate aboveground biomass, the number of quadrats required is 101 and total sampling area is 1.01 ha. When sampling with quadrats sized 100 m \times 100 m, the number of quadrats required is only 4, but total sample area reaches 4 ha (Table 2). When only one quadrat is set, in order to meet the same accuracy, the quadrat area should be 2.25 ha in terms of the basal area, and be 4 ha in terms of aboveground biomass. Therefore, sampling with small quadrats, the spatial variability is greater than that when sampling with large quadrats (Figure 3), and the number of quadrats required is more than that sampling with large quadrats. However, from the perspective of total sampling area, sampling with certain number of small quadrats can achieve higher efficiency (Table 2). As shown in Table 3, when we randomly sample 2, 4 and 6 quadrats with quadrats sized 20 m \times 20 m and 30 m \times 30 m, the estimated values may considerably deviate from the observed values. For instance, when 2 quadrats sized 20 m \times 20 m are used to estimate aboveground biomass, the deviation may reach $\pm 43.7\%$, i.e. the estimated value of biomass may range from 125.57 to 320.49 Mg/ha, and even the deviation may reach $\pm 25.2\%$ when 6 quadrats are randomly sampled (Table 3).

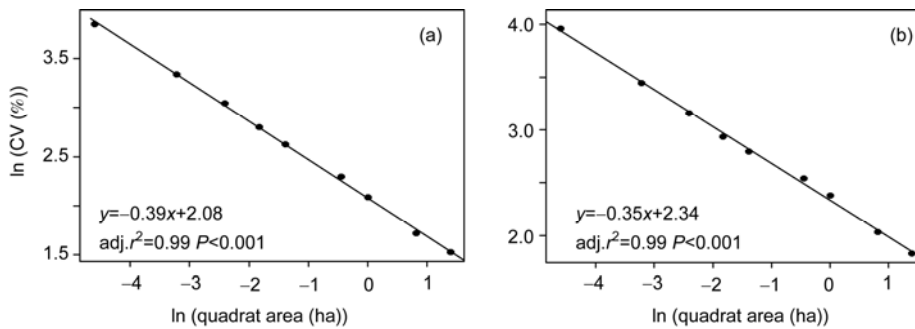


Figure 4 The relationships between quadrat area and coefficients of variation (CV) of basal area (a) and aboveground biomass (b).

Table 2 Required number of quadrats and total sampling area for estimation of basal area and aboveground biomass to within 20% ($\pm 10\%$) of the observed value with 95% confidence for various quadrat sizes

Quadrat area (ha)		0.01	0.04	0.09	0.16	0.25	0.64	1	2.25	4
Basal area	No. of quadrats	86	31	17	11	7	4	3	1	1
	Total area (ha)	0.86	1.24	1.53	1.76	1.75	2.56	3	2.25	4
Aboveground biomass	No. of quadrats	101	36	20	13	10	6	4	2	1
	Total area (ha)	1.01	1.44	1.8	2.08	2.5	3.84	4	4.5	4

Table 3 The error range where the estimated value had 95% probability to fall when randomly sample 2, 4 and 6 quadrats sized 20 m×20 m and 30 m×30 m

Study object	Quadrat area (ha)	No. of quadrats (%)		
		2	4	6
Basal area	0.04	±39.4	±27.9	±22.8
	0.09	±29.3	±20.7	±16.9
Aboveground biomass	0.04	±43.7	±30.9	±25.2
	0.09	±32.9	±23.2	±19.0

3 Discussion

This study analyzed the spatial variability of community structure (basal area and aboveground biomass) in a typical subtropical broad-leaved evergreen forest, and estimated the minimum number of quadrats required for estimation of basal area and aboveground biomass to meet a specified accuracy when random sampling with different sized quadrats. We found that the community structure has great spatial variability (Table 1, Figure 2). The variability of random sampling results were negatively correlated with quadrat area (Figures 3, 4). All of these results are consistent with the studies conducted in tropical and temperate regions [9,13,16]. Although people have realized the variability of community structure and the importance of sampling design [27,28], these issues had been ignored. In the past, many studies only chose a few quadrats with small size or one fixed plot with larger but not big enough plot (such as 1 ha) (Table S1). Our findings suggest that taking full consideration of spatial variability of community structure during the survey, and sampling with higher number of small quadrats will often achieve a higher efficiency (Table 2).

Of course, this study also has some limitations. First, the simulated sampling in this study was conducted in only one place, i.e. a subtropical evergreen broad-leaved forest covering an area of 24 ha. However, the spatial variability of community structure is jointly caused by environment, community process, natural or human disturbance and other factors. Different climate types, different terrain conditions and different degrees of disturbance all can result in differences in community structure [29–31]. As a result, corresponding research carried out in different places can further improve the generalization of the results. Second, both of basal area and aboveground biomass are static parameters of community structure, but the dynamic parameters, such as growth, death and recruitment, are not involved in this study. Monitoring these dynamic parameters is an important approach to study the species coexistence mechanism and the response of forest ecosystems to climate change. Some researchers suggest that compared with static parameters of community structure, the dynamic parameters often shown greater variability [8,9]. Biodiversity monitoring network in

China continues to expand. Currently, nearly 20 forest dynamic plots of 5–25 ha have been established in different places in China, and each of them will be resurveyed every 5 years [32]. Undoubtedly, these survey results will provide the research related to sampling design with data support in a favorable manner.

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Supporting Information

Table S1 The sampling designs of previous studies conducted in subtropical evergreen broad-leaved forests in China

Table S2 Allometric equations used in this study

Table S3 Tree height-DBH regression models used in this study

Text S1 R routine for simulated sampling

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