The influence of quadrat size on spatial heterogeneity of meadow grassland, Northern China

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Abstract. The objective of this study was to determine influence of survey quadrats size on spatial heterogeneity of meadow grassland in north China. An integrative approach was used based on field survey method that the sample line combined with sample quadrat to analyze the vegetation heterogeneity characteristics of Stipa + Leymus Mss. steppe in Hulunbeier grassland. Results showed that: perennial plants, C₃ plants, and cluster grass plants were dominant communities in Hulunbeier grassland under different functional classification. And species richness of them were accounted for the total species richness of more than 86%, 90% and 23.4%, respectively. Despite the species richness of bunch grass plants was little in the functional composition classification, the above ground biomass was accounted for a larger proportion of 50.2%. From the spatial heterogeneity characteristics of Stipa + Leymus Mss. steppe, 6m² is the turning point of the spatial heterogeneity of total species richness, species richness of perennial plant, cluster plant and C₃ plant. That’s because when the quadrat size is less than 6m², the spatial heterogeneity of the total species richness, species richness of perennial plant, cluster plant and C₃ plant were changed significantly with the quadrat size changed, and when the quadrat size is larger than that of 6m², its spatial heterogeneity becomes small. The above analysis shows that in the field survey of Stipa + Leymus Mss. steppe, the quadrat size used in the survey should be not less than 6m², for the summary vegetation information of quadrat size with 6m² can describe the general characteristics of vegetation investigation well.

1. Introduction

In the study of vegetation field survey, the scales are diverse. Different scales correspond with different ecological processes [1]. The relationship between pattern and process, scale character and effect were necessarily evaluation factors [2-4], because the relative degree of the same variables is quite different in distinct scale [5]. How to evaluate the Scale is relative, and the definition of different vegetation representation and different ecological processes for the amplitude of large, medium and
small scale have differences. Generally, it has the rule that a large scale corresponding with long time scales [6].

In researches of species diversity, species are absolutely dependent on space scales [7-9]. In larger space and long time scale, the richness of the species mainly depends on the formation of species rate and extinction rate [10]. In smaller space and short time scale, the amount of species was mainly affected by the birth rates, mortality, the interactions among species and migration rate of species from the communities [11, 12]. Therefore, the study on species composition and structure of the scale dependence is very important, as their results can support to predict species diversity on large scale based on survey data in small scale [13].

Current researches mainly focus on the relationship of species diversity and space scale in the community of forest, grass and etc. [14-17]. Moreover, all studies aim at the spatial scale dependence of some subjects in a particular spatial scope, in order to reveal the ecological significance of the index on the spatial scale [2, 4, 14, 18-20]. Among them, the species composition, structure, pattern and how they change and the relationship between biological diversity involved in different scales, become the hot spot in vegetation ecology research [6]. At the same time, many researchers have noticed another form of scale effect, namely zoning effect. It can result in the change of the definition of the area unit, which leads to the problem of unit area of plasticity [17, 21].

The two aspects involved above were closely related to the investigation of above ground biomass in grassland, Northern China. Up till the present moment, the yield instruction of northern grassland required test quadrats size is 1m² and quadrats number is at least 4 (one of them is for the determination of above ground biomass, the remaining three are used for the measurement of species frequency). And then repeat the investigate method along the transect line or adjacent regions of the sample plots for sampling a certain number of quadrats, and finally 1m² was used to calculate the average amount of above ground biomass. Obviously, this survey method is different from the existing research. The main difference is that all of the existing research is to emphasize the heterogeneity of the vegetation and the relationship between the ecological processes in certain space. And the existing problems during the grassland is no space constraints of the scale of the dependence, so the size of the minimum space scale which can more accurately describe vegetation characteristics is not clear. By far, different scale performance effect expression way of the grass above ground biomass method in China. First, how large is the size of the survey area closer to the real vegetation species, composition of the different functional types and above ground biomass of grassland? Second, how the quadrats with different size affect the result of the survey data in the same vegetation types? Based on the above problems, this paper will analyze the effect of quadrat size on meadow steppe in Hulunbeier grassland to reveal the spatial heterogeneity of species richness and above-ground biomass in meadow steppe.

2. Materials and methods

2.1. Study area

The study area is located in Hulunbeier Plateau, belonging to the evenke county, Inner Mongolia autonomous region, China. Its geographical position is 118 ° 48 '02 "~ 121 ° 09' 25" E, 47 ° 32 '50" ~ 49 15' 37" N. Plot area is 12890 km², accounting for 13.0% of the total area of the Hulunbeier grassland. The climate is temperate continental monsoon climate with long cold winter and short warm summer. The average annual temperature is 2.4 °C to 2.2 °C; The frost-free period 100-120 d; Average annual rainfall is 300 ~ 350 mm, 70% focused on June~ August. The area is in the northwest of Greater Khingan Range hill to Hulunbeier plain transition section. The terrain is tilt from southeast to northwest and the average elevation is 800 ~ 1000m. Vegetation distribution adapts to water spatial variation. From the greater hinggan mountains to the west plateau ridge there are forest, forest steppe, Meadow steppe and typical steppe. The type of zonal soil are chernozem soil and chestnut soil. Due to regional landforms, hydrological and geological effects, it develops implicit area meadow marsh soil, salty meadow soil and sandy soil, etc.
This investigation was conducted in Hulunbeier typical meadow grassland on permanent sample plots of Chinese Research Academy of Environmental Sciences (Figure 1). The vegetation types of permanent sample plots was Stipa + Leymus Mss., Leymus chinensis (Trin.) Tzvel.), and the main plant species were Stipa baicalensis Roshev, Leymus chinensis, Allium anisopodium Ledeb, Cleistogenes squarrosa (Trin.), Agropyron cristatum (Linn.) and miscellaneous class type, etc (Table 1).

2.2. Methods
Field quadrat investigation was conducted during July 10 to July 28, 2016. According to the differences in vegetation composition, three residential areas were set up respectively in the east, middle and west of the sample point and each area with three sample line. In each sample line, according to the difference of the vegetation biomass, 6 (Middle part with relatively small biomass difference) or 8 (East and West part with relatively large biomass difference) quadrats were set up. Investigation includes composition of vegetation species, biomass, height, coverage, abundance, frequency and other vegetation description indicators of each species within the community. The basic area of the investigation was 1 x 1 m². Each quadrat was identified by GPS to calibrate the specific coordinates. Biomass measurement of each species was cut stick to ground and then taken back with a bag after fresh weight, the drying heavy was weighted after drying 10 to 12 h in the 80 °C constant temperature.

2.3. Data analysis
2.3.1. Vegetation classification. Scale dependence characteristics of Stipa + Leymus Mss. was analyzed in three aspects, including plant life form, functional types and ecotype (Table 1). Analysis

Figure 1. Location of study area and quadrat sites
index was the plant species richness and the ground biomass. According to the position of the winter bud, plant life form was classified into annual plant, perennial plant and shrub plant. Plant functional type based on photosynthesis carbon fixation pathway was divided into C\textsubscript{3} and C\textsubscript{4} plant type (CAM recycling plant disappear). The grass was divided into cluster plant and non cluster plant based on the plant ecotype, and it can provide facilitation for analyzing spatial heterogeneity features of cluster plant.

2.3.2. Integration of quadrat size. In the same sampling district (the eastern, middle and western, figure 1), influence of quadrat size changes and combination was in considering while the space relationship among different quadrats was not included. The quadrat size was increased from 1 m\textsuperscript{2} to 16 m\textsuperscript{2} with an interval of 1 m\textsuperscript{2}. The integration of quadrat size was accorded with quadrat investigation numbers. Vegetation composition characteristics analysis accorded with the maximum integration area 16 m\textsuperscript{2}, with the average of three districts. The purpose was to include more plants in order to analyze accurately the vegetation composition.

2.3.3. Spatial heterogeneity described by fractal dimension D. D could be used to describe spatial heterogeneity [22]. D is a non dimensional parameter, therefore, the degree of spatial heterogeneity can be defined through the different variables D comparison [22]. The greater the D was, the higher the spatial heterogeneity was existed in different quadrat size. Vegetation fractal dimension D computation formula is as follows:

$$D = (4 - z)/2$$  \hspace{1cm} (1)

Z is double logarithmic diagram of slope, got through the logarithmic stronghold straight-line fitting. The specific calculation formula is as follows:

$$\log S = \log c + z \log A$$  \hspace{1cm} (2)

S represents the number of species; C represents the number of species on unit area; A is space (quadrat) area; Z is the double logarithmic curve slope of species quantity and the spatial scale (quadrat), the main parameters deciding the relation curve of species diversity and spatial scale [17].

3. Result analysis

3.1. Characteristics of species composition

There were 53 kinds of plant species (Table 1) appearing in the survey site, this survey includes 3 sample districts, 9 sample lines and 64 sample quadrats. According to the classification of plant life form, there were 5 species of annual plants, accounting for 9.4% of the total species; 46 species of perennial plants, accounting for 86.8% of the total species; 2 species of shrub plants, accounting for 3.8% of the total species. According to plant photosynthetic carbon sequestration function classification, there are 49 species of C\textsubscript{3} plants, accounting for 92.5% of the total; 4 species of C\textsubscript{4} plants, accounting for 7.5% of the total species. According to plant ecotype classification, there are 9 species of cluster plant, accounting for 17% of the total species; 44 species of non clumping grass, accounting for 83% of the total species.
| No. | Plants nomenclature                  | Life form | Functional types | Ecotype |
|-----|-------------------------------------|-----------|------------------|---------|
| 1   | Allium anisopodium Ledeb.            | P         | V                | U       |
| 2   | Stipa Baicalensis Roshev.            | P         | V                | C       |
| 3   | Melissilus ruthenicus (L.)           | P         | V                | U       |
| 4   | Agropyron cristatum (Linn.)         | P         | V                | C       |
| 5   | Oxytropis muricata (Pall.)           | P         | V                | U       |
| 6   | Astragalus scaberrimus (Bge.)        | P         | V                | U       |
| 7   | Cleistogenes squarrosa (Trin.)      | P         | O                | C       |
| 8   | Astragalus dahuricus (Pall.)         | P         | V                | U       |
| 9   | Bupleurum chinense DC.               | P         | V                | U       |
| 10  | Carex duriuscula C.A.Mey.            | P         | V                | C       |
| 11  | Cymbaria dahurica                    | P         | V                | U       |
| 12  | Stipa grandis P.                     | P         | V                | C       |
| 13  | Artemisia scoparia                   | A         | V                | U       |
| 14  | Potentilla bifurca L.                | P         | V                | U       |
| 15  | Saposhnikovia diuaria (Turcz.)      | P         | V                | U       |
| 16  | Saussurea japonica                   | P         | V                | U       |
| 17  | Potentilla bifurca var.              | P         | V                | U       |
| 18  | Dostostemon dentatus (Bunge) Ledeb.  | P         | V                | U       |
| 19  | Scorzonera albicaulis Bge.           | P         | V                | U       |
| 20  | Chenopodium glaucum Linn.            | A         | O                | U       |
| 21  | Leymus secalinus (Georgi) Tzvel.    | P         | V                | U       |
| 22  | Euphorbia nematocypya Hand.-Mazz.   | P         | V                | U       |
| 23  | Artemisia frigida Wild.              | S         | V                | U       |
| 24  | Serratula centauroides Linn.         | P         | V                | U       |
| 25  | Iris. Lactea Pall Var Chinensis      | P         | V                | C       |
| 26  | Agropyron mongolicum Keng            | P         | V                | C       |
| 27  | Allium mongolicum Regel              | P         | V                | U       |
| 28  | Clematis hexapetala                  | P         | V                | U       |
| 29  | Galium verum L.                      | P         | V                | U       |
| 30  | Koeleria cristata (L.)               | P         | V                | C       |
| 31  | Euphorbia esula Linn.                | P         | V                | U       |
| 32  | Arctogeton gramineum (Linn.) DC.     | P         | V                | U       |
| 33  | Ostericum sieboldii; (Miq.) Nakai    | P         | V                | U       |
| 34  | Iris. dichotoma                      | P         | V                | U       |
| 35  | Cotried onjaponico Maxim             | A         | O                | U       |
| 36  | Potentilla chinensis Ser.            | P         | V                | U       |
| 37  | Pulsatilla turczaninovi Kryl. et Serg. | P     | V                | U       |
| 38  | Allium tenuissimum Linn.             | P         | V                | U       |
| 39  | Iris. tenuifolin Pall.               | P         | V                | U       |
| 40  | Bupleurum scorzonerifolium Wild.     | P         | V                | U       |
| 41  | Dostostemon micranthus C. A. Mey.    | P         | V                | U       |
| 42  | Caragana microphylia Lam.            | S         | V                | U       |
| 43  | Cymbaria mongolica Maxim             | P         | V                | U       |
| 44  | Potentilla acacilis                  | P         | V                | U       |
| 45  | Leymus chinensis (Trin.) Tzvel.      | P         | V                | U       |
| 46  | Allium ramosum L.                    | P         | V                | U       |
| 47  | Poa sphondylodes Trim. ex Bunge      | P         | V                | U       |
| 48  | Eruoitia ceratoideas (L.) Mey.       | P         | V                | U       |
| 49  | Iris. duthiei                       | P         | V                | U       |
| 50  | Axyris hybrida Linn.                 | A         | V                | U       |
| 51  | Poa annua                           | P         | V                | C       |
| 52  | Thalictrum squarrosus               | P         | V                | U       |
| 53  | Salsola collina Pall.                | A         | O                | U       |
Species richness and biomass on the highest type were selected from different classifications to analyze comparatively in the scale of 16 m² (Fig. 2). The results showed that perennial species richness was 30.7 ± 4 species, accounting for 86.0% of the total species richness (35.7±4.0 species); Above ground biomass was 183.8 ± 22.3g/ m², accounting for 92.3% of the total biomass (199 ± 22.3g/ m²), that indicated the effect of perennial plants in meadow grassland is obvious. Clump grass species richness was only 8.3 ± 0.5 species, accounting for 23.4% of the total species richness, but its above ground biomass had reached 99.9g/ m², accounting for 50.2% of the total biomass, that illustrated the effect of clump grass in the meadow is obvious. Finally, according to C₃ plant characteristics, its species richness was 32.3 ± 4.5, 9 species, accounting for 90.7% of the total species richness; above ground biomass was 166.9 ± 22.3g/ m², accounting for 83.9% of the total biomass, that indicated the effect of C₃ plants in meadow steppe is also obvious.

*Note: the meaning of the capitalized word in the table. A: Annual plant, P: Perennial plant, V: C₃ Plant, O: C₄ plant, C: Cluster plant, U: Not cluster plant

Figure 2. Characteristics of functional classification on different plant species (a. Species richness; b. Above-ground biomass)
3.2. Scale dependent analysis on above-ground biomass
With the increase of the quadrat size, the changes of the above-ground biomass on unit area have changed with varying degrees (Fig. 3). The range of above-ground biomass on unit area was changed from 198.07 to 331.73 g·m⁻². The above-ground biomass of the unit area was significantly higher than that of other sizes when the quadrat size was 1m². Besides, there was a significant decrease in other sizes except size of 15m² with the increase of the quadrat size. There were some differences between the other size, but many of them were not significant different at the 0.05 level.

![Figure 3. Dynamic change of above-ground biomass under different quadrat size](image)

3.3. Analysis of the spatial heterogeneity of species richness
Species richness is the ratio of species numbers in certain quadrat size and total species number in the quadrat size of 16m². The variation range of species richness is 0 to 1. When the number of species is 0 in the quadrat, the species richness in this quadrat is 0, and the species richness in 16m² is 1. With the quadrat size changed from 1m² to 16 m², the species richness showed an increasing trend (Fig. 4). For the C₃ plants, the turning quadrat size for spatial heterogeneity of species richness was 6 m², when the size was less than 6 m², spatial heterogeneity of species richness increased significantly, but the increase was not significant or just increased to a certain extent while the size was larger than 6 m² (Fig. 4a). For the cluster plant, the turning quadrat size for spatial heterogeneity of species richness was 5 m², when the size was less than 5 m², spatial heterogeneity of species richness increased significantly, but the increase was not significant or just increased to a certain extent while the size was larger than 5 m² (Fig. 4b). For the perennial plant, the turning quadrat size for spatial heterogeneity of species richness was 5 m², when the size was less than 5 m², spatial heterogeneity of species richness increased significantly, but the increase was not significant or just increased to a certain extent while the size was larger than 5 m² (Fig. 4c). For the total species richness, the turning quadrat size for spatial heterogeneity of species richness was 6 m², when the size was less than 6 m², spatial heterogeneity of species richness increased significantly, but the increase was not significant or just increased to a certain extent while the size was larger than 6 m² (Fig. 4d). After comprehensive consideration of all species, the quadrat size should not be less than 6 m² for the accuracy of the species richness.
Figure 4. Dynamic changes of the spatial heterogeneity of species richness under different quadrat size in meadow grassland. (a, C3 plant species richness; b, Species richness of cluster plant; c, Species richness of Perennial plant; d, Total species richness
Figure 5. Dynamic changes of the spatial heterogeneity of fractal dimension D under different quadrat size in meadow grassland. (a, D of C₃ plant; b, D of cluster plant; c, D of Perennial plant; d, D of total species.

3.4. Analysis of the spatial heterogeneity of fractal dimension D
D is used to describe the spatial heterogeneity. The greater the D value was, the higher the spatial heterogeneity was caused by the autocorrelation of the space. With the quadrat size changed from 1 m² to 16 m², the fractal dimension D of total species showed a decreasing trend (Fig. 5). For C₃ plants, the turning quadrat size for spatial heterogeneity of fractal dimension D was 6 m², when the size was less than 6 m², spatial heterogeneity of fractal dimension D decreased significantly, but the decrease was not significant or just reached to a certain extent while the size was larger than 6 m² (Fig. 5a). For the cluster plant, the turning quadrat size for spatial heterogeneity of fractal dimension D was 6 m², when the size was less than 6 m², spatial heterogeneity of fractal dimension D decreased significantly, but the decrease was not significant or just reached to a certain extent while the size was larger than 6 m² (Fig. 5b). For the perennial plant, the turning quadrat size for spatial heterogeneity of fractal dimension D was 6 m², when the size was less than 6 m², spatial heterogeneity of fractal dimension D decreased significantly, but the decrease was not significant or just reached to a certain extent while the size was larger than 6 m² (Fig. 5c). For the total species, the turning quadrat size for spatial heterogeneity of fractal dimension D was 6 m², when the size was less than 6 m², spatial heterogeneity of fractal dimension D decreased significantly, but the decrease was not significant or just reached to a certain extent while the size was larger than 6 m² (Fig. 5d). After comprehensive consideration of all
species, the quadrat size should not be less than 6 m$^2$ for the accuracy of the survey of vegetation quadrat.

4. Discussion
Different from all previous researches, scale dependence analysis of this paper didn’t emphasis on scale dependence characteristics of vegetation in a certain space [15-17], but it stressed in results of the influence of the quadrat size on the same vegetation types (group of the cluster plant).

For the scale change features of the vegetation spatial heterogeneity, plant community often can’t be with fully described by one fractal dimension, so according to the characteristics of vegetation changes piecewise fractal analysis [23] was used to get fractal dimension D of different vegetation plant. Piecewise fractal analysis can reflect the hierarchical structure characteristics of vegetation spatial heterogeneity and can also compare spatial heterogeneity in different spaces, which basically can reflect the whole face of the vegetation spatial heterogeneity. This paper analyzed spatial heterogeneity of meadow grassland by fractal dimension D changes.

The heterogeneity characteristics of vegetation is eternity, which can be performed on different scales [5, 24, 25]. When a survey method was used, potential scale effect must be understood by human being. Prior to that, cognition based on the species composition has become the basis of vegetation investigation, but based on the different functional types of vegetation research history is relatively short [26]. From survey results of *Stipa baicalensis* meadow grassland in this paper, perennial plants seem to take absolute superiority, whose species richness account for more than 86% of total species richness and above-ground biomass account for over 92% of the total biomass according to the classification of life forms. And according to the carbon sequestration function classification, $C_3$ plant has dominant proportion, whose species richness account for over 90% of the total species richness and the above ground biomass account for over 83% of the total biomass according to the carbon sequestration function classification. According to the ecological classification, species richness of cluster plant was only account for 23.4% of the total species richness, but its above ground biomass account for 50.2% of the total biomass, indicated cluster plant was the prior species in the meadow steppe.

From the effect of quadrat size on the species richness of $C_3$ plants, perennial plants, cluster plant and total species, the increase rate of plant species richness of *Stipa* meadow grassland significantly reduce when quadrat size was larger than 6 m$^2$; when the quadrat area equal to or less than 6m$^2$, the increase rate was significant. The results indicated that in vegetation investigation of *Stipa* meadow steppe, the quadrat size reflecting the species richness of vegetation should not be less than 6 m$^2$, or there would be deviation to affect different functional types composition of vegetation community.

Slightly different from spatial heterogeneity analysis was intended to reflect the smallest spatial heterogeneity range in basic units (in this article for 1 m$^2$) [23]. Spatial heterogeneity analysis of different functional types shows *Stipa* meadow grassland’s minimum threshold value of spatial heterogeneity was 6m$^2$. The turning quadrat size for spatial heterogeneity of fractal dimension D of $C_3$ plants, perennial plants, cluster plant and total species was 6 m$^2$, when the size was less than 6 m$^2$, spatial heterogeneity of fractal dimension D decreased significantly, but the decrease was not significant or just reached to a certain extent while the size was larger than 6 m$^2$.

5. Conclusion
Based on above analysis, it could be concluded that in the vegetation investigation of *Stipa* meadow steppe, the quadrat size used in the survey should be not less than 6m$^2$, for the summary vegetation information of quadrat size with 6m$^2$ can describe the general characteristics of vegetation investigation well.
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