Population neighborhood density at different scales between grazed and ungrazed communities

XINTING WANG,† CHAO JIANG, FRANK YONGHONG LI, YANG TAI, CUNZHU LIANG, YALI HOU, AND WEIHUA ZHANG

1School of Energy and Power Engineering, Inner Mongolia University of Technology, Hohhot 010051 China
2Institute of Grassland Research, Chinese Academy of Agriculture Sciences, Key of Laboratory of Grassland Ecology and Restoration, Ministry of Agriculture, Hohhot 010010 China
3Ministry of Education Key Laboratory of Ecology and Resource Use of the Mongolian Plateau & Inner Mongolia Key Laboratory of Grassland Ecology, School of Ecology and Environment, Inner Mongolia University, Hohhot 010021 China
4Inner Mongolia Coral Environmental Technology, Hohhot 010010 China

Citation: Wang, X., C. Jiang, F. Yonghong Li, Y. Tai, C. Liang, Y. Hou, and W. Zhang. 2020. Population neighborhood density at different scales between grazed and ungrazed communities. Ecosphere. 00(00):e03275. 10.1002/ecs2.3275

Abstract. It is well recognized that ecological processes are scale-dependent. Despite this recognition, the influence of scale on population density has still not been explored in detail. While the relationship between population density and overall sampling area is well known, understanding how neighborhood density varies with scale is a persistent challenge. Here, neighborhood density refers to the density of individuals occurring within a ring of radius $r$ and width $w$ located at distance $r$ from the typical individual. In this study, we examined the relationship between population neighborhood density and scale. We then compared the results from the O-ring analysis between grazed and ungrazed communities in a temperate steppe ecosystem in China. The primary grazers in this ecosystem are domesticated sheep. Our results show that (1) the relationship between neighborhood density and scale can be well described by the O-ring function; (2) grazing has a substantial impact on neighborhood density and the impact is dependent on spatial scale; specifically, grazing increases the rate of change in neighborhood density across scales and increases plant aggregation at small scales ($<0.5$ m); and (3) the greater aggregation in grazed communities at larger scales can be attributed to habitat heterogeneity, while the greater aggregation at small scales is likely a result of facilitation between individuals. Together, our results demonstrate that understanding neighborhood density across scales is useful for detecting ecological phenomena and for answering important questions in community ecology, while studies without considering scale dependency of neighborhood density will result in spurious or biased conclusions.

Key words: density; neighborhood density; O-ring statistic; scale dependence.

Received 4 February 2020; revised 27 April 2020; accepted 20 May 2020; final version received 31 August 2020.
Corresponding Editor: Sujith Ravi.
Copyright: © 2020 The Authors. This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
† E-mail: wang_x_t2002@163.com

INTRODUCTION

In ecology, population density is an important metric, since it provides clues as to how individuals interact with their local neighbors. Indeed, understanding how population density varies within communities is critical for studying important processes such as density dependence (Lin et al. 2012, Zhu et al. 2015, Fernández-Bellon et al. 2016), population control (Dahlgren et al. 2016), and interactions between predators and their prey (Mayor and Schaefer 2005). However, population density is most often measured as a single mean-field value that is highly dependent
on the sampling area (e.g., Yoda et al. 1963, Miyanishi et al. 1979, Westoby 1984, Enquist et al. 1998, Niklas et al. 2003, Gunton and Kunin 2007, 2009, Li et al. 2013), even though it is well known that population density can vary within a single population (Smallwood and Schonewald 1996, Gaston et al. 1999, Pautasso and Weisberg 2008). In other words, population density is scale-dependent. Importantly, studies that fail to take into account the scale dependency of population density will result in spurious or biased conclusions (Heads and Lawton 1983, Ray and Hastings 1996, Hixon et al. 2002). These studies show that it is necessary to consider the scale dependency of measures of population density. Therefore, exploring variation in population density at multiple spatial scales is of fundamental importance for investigation, especially in sessile organisms such as plants and many insect larvae (Rothman and Darling 1990, Ray and Hastings 1996, Gunton and Kunin 2007, 2009).

The scale dependency of population density has received considerable attention in the literature (Matter 2000, Gunton and Kunin 2007, 2009). In particular, the influence of sampling area on population density has been studied from a number of different perspectives (Connor et al. 2000, Mayor and Schaefer 2005, Gunton and Kunin 2007). As a result of this work, three primary hypotheses have emerged for explaining this important relationship. The first hypothesis predicts that population density stays the same regardless of sampling extent (MacArthur and Wilson 1967). This is due to the fact that species richness and abundance both increase as a function of sampling area (MacArthur and Wilson 1967). The second hypothesis states that population density increases with sampling area due to expectations derived from the patch individual–area relationship theory (Gaston and Matter 2002). This theory postulates that resource availability increases with area while predator efficiency declines, resulting in overall higher densities (Root 1973). Lastly, density may be expected to decline with area, as predicted by the generalized individual–area relationship (Gaston and Matter 2002). These declines may result from density compensation (Schoener 1986) or from arbitrary sampling by centering high-abundance sites (Smallwood and Schonewald 1996).

Each of the above hypotheses has been supported empirically (Mayor and Schaefer 2005). While the overall relationship between population density and area has been explored (Matter 2000, Gaston and Matter 2002), understanding the relationship between population density and spatial scale still remains unclear. For example, within a population, different individuals can be found within patches of variable size, resulting in neighborhood density values that are scale-dependent. This scale dependency can be quantified by examining the mean number of neighbors within rings of variable radius \( \rho \) (Wiegand and Moloney 2004). Therefore, neighborhood density refers to the density of individuals occurring within a ring of radius \( \rho \) and width \( w \) located at distance \( r \) from the typical individual. In contrast to mean-field estimates of population density, this method can better account for the spatial distribution of individuals in the population. This can be highly useful for uncovering the processes influencing neighborhood density. For example, competition for water between plants may be strongest at some characteristic distance, \( r \), from a focal plant. In contrast, estimating population density within a single sampling area masks these scale dependencies, potentially leading to incomplete conclusions about the intensity of competition in certain locations. However, in previous studies, almost no attention has been given to testing neighborhood densities across a range of scales. Therefore, it is a new challenge to testing the change in neighborhood density with scale.

Here, we examine the scale dependency of neighborhood density within a temperate steppe ecosystem in China. Importantly, these ecosystems have been highly disturbed by overgrazing of domesticated sheep since the 1980s (Li et al. 2008, Wang et al. 2015). Chronic herbivory is known to change overall population densities, for example, the density of unpalatable species increases, while the density of palatable species decreases (Wang et al. 1999, Wang and Jiang 2018). However, it is not well known whether chronic herbivory alters plant neighborhood density at different spatial scales, which is important for understanding the responses of plant communities to long-term overgrazing in the steppe ecosystems. Statistical methods that allow for the observation of changes in neighborhood densities across scales can be highly useful in this regard (Wiegand and Moloney 2014).
The first goal of this study was to document neighborhood density across scales and compare estimates between grazed and ungrazed communities. The second goal was to identify the processes that grazing influences the relationship between neighborhood density and scale. For the second goal, we examine two corresponding hypotheses and predictions. First, we hypothesize (H1) that herbivores create habitat heterogeneity via grazing and predict that greater aggregation among individuals will be observed at larger spatial scales in the grazed communities than in the ungrazed communities. This is because habitat heterogeneity can bring about individual aggregation at larger scales (Wiegand et al. 2007). An alternative hypothesis (H2) is that herbivores shift the dominant processes affecting plant spatial distributions to facilitation. As such, we predict that aggregation in grazed communities will be greater at smaller scales relative to the ungrazed communities, as facilitation can cause individual clustering at small scales (Graff and Aguiar 2011). Identifying support for either of these hypotheses will help us determine how grazing influences the scale dependency of neighborhood density in grassland ecosystems.

**Materials and Methods**

**Study sites and objective species**

Data were collected from a permanent field site within the Inner Mongolia Grassland Ecosystem Research Station (IMGERS, 116°42’ E, 43°38’ N). The station is located within the Xilin River Basin in the Inner Mongolia Autonomous Region of China (Bai et al. 2004). In the region, dark chestnut soils with a humus layer of 20–30 cm thick and a calcic layer below 50–60 cm are usually present. Average annual temperature is around 0°C, and the mean annual precipitation is around 350 mm. Roughly 60–80% of annual rainfall occurs between June and August (Li et al. 2008). Annual potential evaporation ranges from 1600 to 1800 mm (Niu et al. 2000). The vegetation in the region is the steppes dominated by *Leymus chinensis* and *Stipa grandis* (Wang et al. 1985), the biomass of these two species accounting for 25–50% of the total biomass. Species richness of the sites ranges from 31 to 44 species (Chen et al. 1983, Wang and Jiang 2018). The growing season starts in early April and runs to late September for the perennial plant species. Annual plants typically germinate in early July following heavy rains (Bai et al. 2004).

By the 1980s, the region had become heavily disturbed by long-term overgrazing by sheep (Wang et al. 1996a, b, 2000a, b, Li et al. 2008). Previous studies have shown that these seriously degraded communities commonly contain plants with shortened internodes, smaller leaf blades, and shallower root systems (Wang et al. 2000a, b). A precipitous decline in overall biomass and productivity of these grassland ecosystems has also been observed as a direct result of overgrazing (Wang et al. 1996a).

For vegetation sampling, we chose three sites with relatively distinct species compositions. The plant community types at these sites represent the most widely distributed grassland communities across the Eurasia steppe region (Wang et al. 1985). Site A is dominated by a perennial rhizome grass *L. chinensis*, while Site B is dominated by a perennial bunchgrass *S. grandis*. At both sites, an area of 500 × 500 m in dimension has been fenced since 1979 to exclude animal grazing. At the time of setting up the exclosure, both sites were considered to be in excellent condition and representative of undisturbed, climax steppe communities (Bai et al. 2004). The immediate area outside of both sites is open to large animal grazing and has become seriously degraded. Site C is 500 × 50 m in dimension, is co-dominated by *L. chinensis* and *S. grandis*, and has been fenced since 1996 to exclude animal grazing. When Site C was fenced, it was already seriously degraded, and the areas outside the fence remained seriously degraded today. We chose *L. chinensis* as objective species for Site A, chose *S. grandis* as objective species for Site B, and chose *L. chinensis* and *S. grandis* as objective species for Site C.

**Field sampling**

The studies were conducted in July 2004 for Site C and in July 2017 for Sites A and B. Within each site, three 5 × 5 m replicate community blocks were chosen. In each of the three replicate blocks, the number of individuals of the target species was recorded (Tables 1, 2, and 3), and the locations of all individuals were recorded using the new digital method of data collection described by Wang et al. (2020). At each site and
for each of the three replicates, a flat 5 × 5 m sampling area was chosen so as to avoid abiotic heterogeneity. The 5 × 5 m plots were then divided into 100 subplots of 50 × 50 cm using small wooden stakes. The vegetation within each 0.25-m² subplot was then photographed using a digital camera that was mounted to a telescoping extension pole. Pictures were taken from 1.75 m above the ground at an approximate downward angle of 90° using a fixed infinite focus lens. Automatic camera settings were used. Whole-plot pictures were then used to map the spatial locations of all individuals in the plots. Images were then used to identify all individuals to species.

**Data analysis**

**O-ring function.**—We used the O-ring statistic, O(r), to examine the scale dependency of neighborhood density. The O-ring statistic is closely related to the pair correlation function g(r), where O(r) = λg(r) (Wiegand and Moloney 2004). The pair correlation function g(r) is derived from the K(r) function, where g(r) = (2πr)^{-1}dK(r)/dr (Wiegand and Moloney 2014). Ripley’s K(r) function (Ripley 1976, 1977, 1981) is based on the distribution of distances between pairs of points and is commonly used to describe the structure of spatial point patterns (Wiegand and Moloney 2004). The λK(r) gives the expected number of points found within a distance r of an arbitrarily

| Community state |  
|-----------------|
| Plot | Number in each plot | Density in each plot (plants/m²) | Overall density† (plants/m²) |
|---|---|---|---|
| Grazed | G1 | 3447 | 137.88 | 125.76 ± 12.08* |
| Community | G2 | 3142 | 125.68 |
| | G3 | 2843 | 113.72 |
| Ungrazed | U1 | 2969 | 118.76 | 131.26 ± 22.36* |
| Community | U2 | 2949 | 117.96 |
| | U3 | 3927 | 157.08 |

† Combining three plots.

* Nonsignificant difference P = 0.726 > 0.05.

**Table 2. Stem count and density of *S. grandis* in the 5 × 5 m study plots between grazed and ungrazed conditions in the *S. grandis* community (Site B).**

| Community state |  
|-----------------|
| Plot | Number in each plot | Density in each plot (plants/m²) | Overall density† (plants/m²) |
|---|---|---|---|
| Grazed | G1 | 1383 | 55.32 | 52.83 ± 1.79* |
| Community | G2 | 1299 | 51.96 |
| | G3 | 1280 | 51.20 |
| Ungrazed | U1 | 1011 | 40.44 | 39.11 ± 1.27* |
| Community | U2 | 974 | 38.96 |
| | U3 | 948 | 37.92 |

† Combining three plots.

* Significant difference P = 0.002 < 0.05.

**Table 3. Stem count and density of *L. chinensis* and *S. grandis* in the 5 × 5 m study plots between grazed and ungrazed treatments in the *L. chinensis* and *S. grandis* community (Site C).**

| Species | Community state | Plot | Number in each plot | Density in each plot (plants/m²) | Overall density† (plants/m²) |
|---|---|---|---|---|---|
| *L. chinensis* | Grazed community | G1 | 1428 | 57.12 | 53.95 ± 11.33* |
| | G2 | 1649 | 65.96 |
| | G3 | 969 | 38.76 |
| Ungrazed community | U1 | 2023 | 80.92 | 78.16 ± 2.56* |
| | U2 | 1869 | 74.76 |
| | U3 | 1970 | 78.80 |
| *S. grandis* | Grazed community | G1 | 169 | 6.76 | 6.52 ± 0.80** |
| | G2 | 184 | 7.36 |
| | G3 | 136 | 5.44 |
| Ungrazed community | U1 | 427 | 17.08 | 19.56 ± 1.77** |
| | U2 | 512 | 20.48 |
| | U3 | 528 | 21.12 |

† Combining three plots.

* Significant difference P = 0.042 < 0.05.

** Significant difference P = 0.014 < 0.05.
The O-ring statistic estimates the mean number of neighboring individuals within an annulus of radius \( r \) and width \( w \) around a typical point of the pattern (Wiegand and Moloney 2004; Fig. 1). As a result, the function has the characteristic advantage of being a scale-dependent neighborhood density function. In other words, it describes the relationship between neighborhood density and spatial scale (Galiano 1982, Wiegand and Moloney 2014). The function is defined as

\[
O^w(r) = \frac{\frac{1}{n} \sum_{i=1}^{n} \text{Points}[R^w_i(r)]}{\frac{1}{n} \sum_{i=1}^{n} \text{Area}[R^w_i(r)]}
\]

(1)

where \( n \) is the number of individuals within the point pattern, and the operator \( R^w_i(r) \) is the annulus with radius \( r \) and width \( w \) centered on the \( i \)th plant within the sampling area. The operator \( \text{Points}[X] \) counts the number of points in region \( X \) within the sampling area, and the operator \( \text{Area}[X] \) represents the area of region \( X \).

For the O-ring statistic, \( O(r) \), a single weighted O-ring statistic for \( N \) replicate plots, can be obtained by extending the one replicate formula (Eq. 1) as such:

\[
O^w(r) = \frac{\sum_{j=1}^{N} \sum_{i=1}^{n_j} \text{Points}[R^w_i(r)] + \cdots + \sum_{j=1}^{N} \sum_{i=1}^{n_j} \text{Points}[R^w_i(r)]}{\sum_{j=1}^{N} \sum_{i=1}^{n_j} \text{Area}[R^w_i(r)] + \cdots + \sum_{j=1}^{N} \sum_{i=1}^{n_j} \text{Area}[R^w_i(r)]}
\]

(2)

here, \( i \) is the \( i \)th individual of plant in replicate plot \( j \), \( n_j \) is the individual number of plant in replicate plot \( j \), and \( N \) is the number of replicates.

**Null models**

Unbiased interpretations of the O-ring statistic require the selection of an appropriate null
model that addresses the specific biological question being asked (Wiegand and Moloney 2004). For the second goal, we chose the homogenous Poisson process and the heterogeneous Poisson model. We used the homogenous Poisson process to test the effects of aggregation between grazed and ungrazed communities. We then used the heterogeneous Poisson model to distinguish the individual effects of facilitation from habitat heterogeneity. The two null models were described as follows.

The homogenous Poisson process implies that the intensity, \( \lambda \), does not vary across the study region. In other words, the probability of finding \( k \) points within area \( w \) follows a Poisson distribution. Thus, any point within the pattern has an equal probability of occurring at any position in the study region, and the position of any point is independent of the position of any other point.

For the heterogeneous Poisson model, the density of points in the pattern is associated with certain environmental factors. Compared with the homogenous Poisson process, the constant intensity is replaced by a function \( \lambda(x, y) \) that varies with location \( (x, y) \). Here, nonparametric methods are used to estimate the intensity function \( \lambda(x, y) \) directly from the data using smoothing techniques based on kernel estimators (Wiegand and Moloney 2014). From observations made in the field prior to this study, we found that environmental conditions in the grazing community varied across scales of about 2.0 m (personal observation). Thus, we constructed the intensity function \( \lambda(x, y) \) using a bandwidth \( R = 1.0 \) m. The data on environmental gradients would help us understand the role that habitat heterogeneity plays in driving the spatial patterns of our study species. However, this also was not the primary focus of our work, and we thus use the heterogeneous Poisson process by nonparametric methods to infer these processes. This strategy is particularly appropriate for situations in which no additional information on environmental variables is available, as it allows for the detection of potential gradients in the intensity of points to underlying heterogeneity (Wiegand and Moloney 2014).

In our study, we combined the three replicates in each grazing condition into a single weighted the O-ring statistic (Diggle 2013, Wiegand and Moloney 2014). Because the number of individuals was different between grazed and ungrazed treatments in each site (Table 1, 2, and 3), we compared the degree of aggregation between grazed and ungrazed conditions using the standardization described by Graff and Aguiar (2011). This method involves calculating the difference between the weighted O(r) statistic values and the upper (or lower) confidence limits for the weighted values that show a positive or negative deviation from the null model. The formulation for the confidence intervals is as follows:

\[
\text{If } O(r) \text{ > upper 99% confidence limit (CL}^+\text{)} \text{ then } y = (O(r) - CL^+) / CL^+ > 0
\]
\[
\text{If } O(r) \text{ < upper 99% confidence limit (CL}^+\text{)} \text{ and } O(r) \text{ > lower 99% confidence limit (CL}^-\text{)}, \text{ then } y = 0
\]
\[
\text{If } O(r) \text{ < lower 99% confidence limit (CL}^-\text{)}, \text{ then } y = (O(r) - CL^-) / CL^- < 0
\]

Next, we calculated the aggregation effects between grazed and ungrazed conditions for each distance class (i.e., from 0 to 2.5 m by increments by 0.1 m). When using the homogenous and heterogeneous Poisson process, positive values indicate clustered spatial patterns, while negative values indicate overdispersed spatial patterns.

All analyses were conducted using Programita version 2014 (Wiegand and Moloney 2014). We used this program to compare our observed data with the null models described above, and confidence envelopes were constructed using the highest and lowest O(r) from 99 replicates of the null model. The ring width \( w \) was 0.1 m in our study.

**RESULTS**

**Neighborhood density varies with scale between grazed vs ungrazed community**

At Site A, the neighborhood density of \( L. chinensis \) decreased with distance and then leveled off in both grazed and ungrazed plots (Fig. 2A), but with a greater decreasing rate under grazed than non-grazed conditions. The neighborhood density of \( L. chinensis \) was overall smaller under grazed than non-grazed conditions (Fig. 2A).

At Site B, the neighborhood density of \( S. grandis \) decreased with scale and then plateaued under grazed conditions but increased with scale and then plateaued under ungrazed conditions.
Neighborhood density of *S. grandis* was generally greater under grazed than ungrazed conditions across the scales (Fig. 2B). Neighborhood density of *S. grandis* was higher in the grazed conditions than in the ungrazed conditions at 0.0–0.15 m (Fig. 2D), and the decreasing rate was greater under grazed than non-grazed conditions at scales < 0.5 m. Neighborhood density of this species was higher in the grazed than the ungrazed conditions at 0.0–0.15 m (Fig. 2D).

At Site C, the neighborhood density of *L. chinensis* decreased with increasing spatial scale and then leveled off under both grazed and ungrazed conditions (Fig. 2C), and the decreasing rate was greater at scales <0.5 m under grazed than non-grazed conditions. Neighborhood density of *L. chinensis* was higher in the grazed conditions than in the ungrazed conditions at 0.0–0.19 m (Fig. 2C). Similarly, the neighborhood density of *S. grandis* declined with increasing spatial scale and then leveled off under both grazed and ungrazed conditions (Fig. 2D), and the decreasing rate was greater under grazed than non-grazed conditions at scales < 0.5 m. Neighborhood density of this species was higher in the grazed than the ungrazed conditions at 0.0–0.15 m (Fig. 2D).

The effect of habitat heterogeneity and facilitation on neighborhood density between ungrazed and grazed community

Intraspecific aggregation based on the homogeneous Poisson process.—At Site A, individuals of *L. chinensis* were clustered from 0.0- to 2.5-m scales in the grazed communities and at 0.0- to 0.7-m scales in the ungrazed communities (Fig. 3)
The degree of aggregation of *L. chinensis* was greater in the grazed than the ungrazed sites at all spatial scales (Fig. 3A).

At Site B, individuals of *S. grandis* were clustered at 0.0–1.3 m in the grazed community and were overdispersed at 0.0–0.2 m in the non-grazed community (Fig. 3B). The degree of aggregation was greater under grazed than non-grazed conditions between 0.0 and 1.3 m (Fig. 3B).

At Site C, individuals of *L. chinensis* were clustered at 0.0–1.9 m under grazed condition and at 0.0–2.5 m under ungrazed condition (Fig. 3C). The degree of aggregation was higher in grazed than ungrazed communities at 0.0–0.5 m and 0.6–1.1 m for *L. chinensis* (Fig. 3C). Similarly, individuals of *S. grandis* were clustered at 0.0–2.0 m scales under grazed condition and at 0.0–0.9 m scales under ungrazed condition (Fig. 3D). The degree of aggregation was greater in grazed than ungrazed communities at 0.0–0.5 m and 0.8–2.0 m for *S. grandis* (Fig. 3D).

**The effect of habitat heterogeneity and facilitation on intraspecific aggregation.**—At Site A, *L. chinensis* individuals were clustered according to the heterogeneous Poisson process between 0.0 and 0.70 m in the grazed community and between 0.8 and 2.5 m in the ungrazed community (Fig. 3A).

**Fig. 3.** Results from the weighted O-ring function analysis for grazed and ungrazed communities. Data are expressed as (O(r)-CL)/CL, and the 99% confidence limits (CL) were constructed using the highest and lowest O (r) from 99 replicates of the homogeneous Poisson model (CSR). (A) *Leymus chinensis* in the *L. chinensis* community (Site A); (B) *Stipa grandis* in the *S. grandis* community (Site B); (C) *Leymus chinensis* in the *L. chinensis* and *S. grandis* community (Site C); and (D) *Stipa grandis* in the *L. chinensis* and *S. grandis* community (Site C).
0.0 and 0.40 m in the ungrazed community (Fig. 4A). Greater aggregation was also observed between 0.0 and 0.7 m in the grazed than the ungrazed community (Fig. 4A).

At Site B, individuals of *S. grandis* were clustered according to the heterogeneous Poisson process at 0.0–0.20 m in the grazed community, and more aggregation occurred at 0.0–0.2 m in the grazed than the ungrazed community (Fig. 4B).

Finally, at Site C, individuals of *L. chinensis* clustered according to the heterogeneous Poisson process at 0.0–0.40 m in the grazed community and at 0.0–0.50 m in the ungrazed community (Fig. 4C). Similarly, individuals of *S. grandis* clustered at 0.0–0.40 m in the grazed community and at 0.0–0.30 m in the ungrazed community (Fig. 4D). More aggregation occurred at small scales in the grazed than in the ungrazed community at 0.0–0.35 m for *L. chinensis* (Fig. 4C) and at 0.0–0.4 m for *S. grandis* (Fig. 4D).

### Population density between grazed and ungrazed communities

At Site A, overall population density of *L. chinensis* was lower in the grazed conditions (125.76 ± 12.08 plants/m²) than in the ungrazed conditions (131.26 ± 22.36 plants/m²), although the differences were not significant (*P* = 0.726, *t* = 0.62, *d.f.* = 17).

---

Fig. 4. Results from the weighted O-ring function analysis for grazed and ungrazed communities. Data are expressed as (O(r)-CL)/CL, and the 99% confidence limits (CL) were constructed using the highest and lowest O(r) from 99 replicates of the heterogeneous Poisson model. (A) *Leymus chinensis* in the *L. chinensis* community (Site A); (B) *Stipa grandis* in the *S. grandis* community (Site B); (C) *Leymus chinensis* in the *L. chinensis* and *S. grandis* community (Site C); and (D) *Stipa grandis* in the *L. chinensis* and *S. grandis* community (Site C).
Table 1). At Site B, population density of *S. grandis* was significantly higher in the grazed (52.83 ± 1.79 plants/m²) than the ungrazed conditions (39.11 ± 1.27 plants/m²; *P* = 0.002, Table 2). At Site C, population density of *L. chinensis* was significantly lower in the grazed community (53.95 ± 11.33 plants/m²) than the ungrazed community (78.16 ± 2.56 plants/m²; *P* = 0.042, Table 3). Similarly, population density of *S. grandis* was significantly lower in the grazed community (6.52 ± 0.80 plants/m²) than the ungrazed community (19.56 ± 1.77 plants/m²; *P* = 0.014, Table 3).

**Discussion**

*The relationship between neighborhood density and spatial scale in grazed and ungrazed communities*

The first goal of our study was to document neighborhood density across scales. Our results show that the relationship between neighborhood density and scale can be well described using O-ring function (Fig. 2). Our findings provide evidence that grazing has a substantial impact on neighborhood density and that these effects are dependent on spatial scale (Fig. 2). Specifically, the degree of aggregation of *L. chinensis* individuals was greater under grazed than ungrazed conditions at all spatial scales at Site A (Fig. 3A). The degree of aggregation of *S. grandis* was greater under grazed than non-grazed conditions between 0.0 and 1.3 m at Site B (Fig. 3B). For *L. chinensis*, the degree of aggregation was higher in grazed plots at 0.0–0.5 m (Fig. 3C). For *S. grandis* at Site C, aggregation was higher in grazed plots between 0.0–0.5 m and 0.8–2.0 m (Fig. 3D).

The second goal of our study was to identify how grazing influences the scale dependency of population density. By using the homogenous Poisson process, we found that greater aggregation occurred under grazed than non-grazed conditions (Fig. 3). Greater aggregation in grazed communities may be the result of habitat heterogeneity or facilitation, because habitat heterogeneity can cause individual aggregation at larger scales (Wiegand et al. 2007), while facilitation can bring about individual clustering at small scales (Graff and Aguiar 2011). We sought to disentangle the effects of habitat association and facilitation by applying the heterogeneous Poisson process. Once we corrected for environmental heterogeneity, any aggregation effects beyond 0.5 m in scale were virtually absent (Fig. 4), suggesting that aggregation at neighborhoods >0.5 m is due to habitat heterogeneity. These results suggest that greater aggregation at larger scales (Fig. 3) is most likely a result of grazing enhancement to habitat heterogeneity, most likely in soil nutrients, under grazed conditions (Yang et al. 2016). This result supports our first hypothesis (H1). However, further experimental work is necessary to confirm this.

We also found that aggregation at neighborhoods was greater at small scales in grazed sites when using the heterogeneous Poisson process (Fig. 4). These results suggest that greater aggregation at these small scales cannot be attributed to habitat heterogeneity in grazed sites. In our study, grazed vs. ungrazed sites can be viewed as stressful vs. unstressful abiotic conditions, because the region has been heavily disturbed by long-term overgrazing by sheep (Wang et al. 1996a). Long-term overgrazing can result in miniaturization of plant individuals, characterized by shortened internodes, contracted leaf blades, and shallowly distributed roots (Wang et al. 2000b); and lead to a substantial reduction in plant community production (Wang and Jiang 2018). Facilitation is expected to be more intense than competition when abiotic stress or consumer pressure is high (i.e., Bertness and Callaway 1994, Callaway 2007, Kikvidze et al. 2011). For example, the stress gradient hypothesis (SGH) postulates that the relative importance of facilitation compared to competition increases with abiotic stress or consumer pressure (Bertness and Callaway 1994, Callaway and Walker 1997). Specifically, the SGH predicts that facilitative interactions dominate under high-stress conditions, and competitive interactions dominate in low-stress conditions. As a result, greater aggregation at small scales and in grazed sites can be a result of facilitation between individuals, and these findings are consistent with our second hypothesis (H2). Facilitation at small scales may be due to trampling by herbivores (Wang and Jiang 2018). In our study, plant species are subject to constant trampling by long-term overgrazing. In order to defend against constant trampling, individuals are expected to cluster together at small scales (Wang et al. 2011). Therefore, our
results suggest that facilitation is an effective mechanism for resisting overgrazing in grazed communities. These findings are important for understanding the mechanisms of grazing-induced grassland degradation and for developing conservation and management plans for the steppe ecosystems. For the conservation and management of steppe ecosystems in China, grazing intensity should be reduced by managing grazing livestock.

As mentioned above, we described how grazing might influence the dynamic relationships between neighborhood density and scale using the heterogeneous Poisson model. However, there are limitations in exploring the mechanism for the changes in population neighborhood density through the use of null model, since spatial statistical techniques based on observational data cannot suggest causal relationships (Murrell et al. 2001). In other words, different ecological processes can result in the same spatial pattern (Wiegand and Moloney 2004). As such, further experimental work is necessary for manipulating these different processes.

To quantify the change in neighborhood density with scale, we offer two recommendations. The first is on the size of sampling area. We know that the appropriate size of the sampling area varies with the studied population and ecosystems and with research objectives. Our results demonstrate that the sampling area of 5 x 5 m is appropriate for quantifying the change in neighborhood density with scale for the studied population in steppe ecosystem, as the sampled data have objectively described the relationship between population neighborhood density and scale under grazed vs. ungrazed conditions. The second recommendation is on the choice of the width of the ring w. We know that the neighborhood density is dependent on spatial scale r according to the definition, that is, the neighborhood density can only be estimated at specific scales. The width of the ring w greatly affects the estimated neighborhood density; the use of too narrow rings will produce jagged plots of the curve on the neighborhood density and scale, and make them difficult to interpret. Therefore, the width of the ring should be carefully chosen to quantify the relationship between neighborhood density and scale.

Population density in grazed and ungrazed communities

In our study, we compared population densities between grazed and ungrazed sites. We found that variation in population density was not consistent for grazed vs. ungrazed plots among the different communities. For example, in the L. chinensis community, the density of L. chinensis was not significantly different between grazed and non-grazed sites (Table 1), while in the S. grandis community, the density of S. grandis was higher in the grazed site than in the ungrazed site (Table 2). In the L. chinensis and S. grandis community, the densities of L. chinensis and S. grandis were lower in the grazed site than in the ungrazed site (Table 3). These results suggest that large-scale effects of grazing on mean density (Table 1, 2, and 3) cannot predict the patterns we found here by looking at the scale-specific neighborhood density relationships (Fig. 2). This is likely due to the fact that population density describes the average value under a specific sampling area, while ignoring the spatial distribution of individuals in the population. In contrast, estimating population neighborhood density at different spatial scales is more related to the spatial distribution of individuals in the population. Therefore, thinking about neighborhood density at different spatial scales is valuable for detecting ecological phenomena and answering important ecological questions.

In summary, our research illustrates that population neighborhood density changes with spatial scale in plant communities. The differences in neighborhood densities at different spatial scales have widespread implications for population dynamics, community-level patterns, and density dependence. Moreover, our findings are important for understanding the mechanisms underlying grazing-induced grassland degradation and for developing conservation and management plans for steppe ecosystems in China and elsewhere. Ultimately, we hope that our research inspires other ecologists to recognize the importance of the scale dependency of neighborhood density.

ACKNOWLEDGMENTS

This research was supported by the National Natural Science Foundation of China (32060251), the Natural Science Foundation of Inner Mongolia (2017MS0302),
the Basic Research Expenses Special Funds of the Central Public Welfare Scientific Research Institutes (1610332016002), and the Natural Science Foundation of Inner Mongolia University of Technology (ZD201403). We would like to thank Stephen Murphy at Yale University for his assistance with the English language and grammatical editing of the manuscript.

**LITERATURE CITED**

Bai, Y., X. Han, J. Wu, Z. Chen, and L. Li. 2004. Ecosystem stability and compensatory effects in the Inner Mongolia grassland. Nature 431:181–184.

Bertness, M. D., and R. M. Callaway. 1994. Positive interactions in communities. Trends in Ecology and Evolution 9:191–193.

Callaway, R. M. 2007. Positive interactions and interdependence in plant communities. Springer, Dordrecht, The Netherlands.

Callaway, R. M., and L. R. Walker. 1997. Competition and facilitation: a synthetic approach to interactions in plant communities. Ecology 78:1958–1965.

Chen, Z., D. Huang, and H. Zhang. 1983. The reserve and distribution of nitrogen in the *Leymus* and *Stipa* grassland. Chinese Journal of Plant Ecology 7:143–151.

Connor, E. F., A. C. Courtney, and J. M. Yoder. 2000. Individuals-area relationship: the relationship between animal population density and area. Ecology 81:734–738.

Dahlgren, J. P., K. Bengtsson, and J. Ehrlén. 2016. The demography of climate-driven and density-regulated population dynamics in a perennial plant. Ecology 97:899–907.

Diggle, P. J. 2013. Statistical analysis of spatial and spatio-temporal point patterns. Third edition. CRC Press, Boca Raton, Florida, USA.

Enquist, B. J., J. H. Brown, and G. B. West. 1998. Allometric scaling of plant energetics and population density. Nature 395:163–165.

Fernández-Bellon, D., A. Cortés-Avizanda, R. Arenas, and J. A. Donázar. 2016. Density-dependent productivity in a colonial vulture at two spatial scales. Ecology 97:406–416.

Galiano, E. F. 1982. Pattern detection in plant populations through the analysis of plant-to-all-plants distances. Vegetatio 49:39–43.

Gaston, K. J., T. M. Blackburn, and R. D. Gregory. 1999. Does variation in census area confound density comparisons? Journal of Applied Ecology 36:191–204.

Gaston, K. J., and S. F. Matter. 2002. Individuals-area relationships: comment. Ecology 81:734–748.

Graff, P., and M. R. Aguiar. 2011. Testing the role of biotic stress in the stress gradient hypothesis. Processes and patterns in arid rangelands. Oikos 120:1023–1030.

Gunton, R. M., and W. E. Kunin. 2007. Density effects at multiple scales in an experimental plant population. Journal of Ecology 95:435–445.

Gunton, R. M., and W. E. Kunin. 2009. Density-dependence at multiple scales in experimental and natural plant populations. Journal of Ecology 97:567–580.

Heads, P. A., and J. H. Lawton. 1983. Study on the natural enemy complex of the holly leaf-miner: the effects of scale on the detection of aggregative responses and the implications for biological control. Oikos 40:267–276.

Hixon, M. A., S. W. Pacala, and S. A. Sandin. 2002. Population regulation: historical context and contemporary challenges of open vs. closed systems. Ecology 83:1490–1508.

Kikvidze, Z., M. Suzuki, and R. Broocker. 2011. Importance versus intensity of ecological effects: Why context matters. Trends in Ecology and Evolution 26:383–388.

Li, L., J. Weiner, D. Zhou, Y. Huang, and L. Sheng. 2013. Initial density affects biomass-density and allometric relationships in self-thinning populations of *Fagopyrum esculentum*. Journal of Ecology 101:475–483.

Li, Y., W. Wang, Z. Liu, and S. Jiang. 2008. Grazing gradient versus restoration succession of *Leymus chinensis* (*Trin.*) *Tzvel.* Grassland in Inner Mongolia. Restoration Ecology 16:572–583.

Lin, L., L. S. Comita, Z. Zheng, and M. Cao. 2012. Seasonal differentiation in density-dependent seedling survival in a tropical rain forest. Journal of Ecology 100:905–914.

MacArthur, R. H., and E. O. Wilson. 1967. The theory of island biogeography. Princeton University Press, Princeton, New Jersey, USA.

Matter, S. F. 2000. The importance of the relationship between population density and habitat area. Oikos 89:613–619.

Mayor, S. J., and J. A. Schaefer. 2005. The many faces of population density. Oecologia 145:276–281.

Miyaniishi, K., A. R. Hoy, and P. B. Cavers. 1979. A generalized law of self-thinning in plants. Journal of Theoretical Biology 78:439–442.

Murrell, D. J., D. W. Purves, and R. Law. 2001. Uniting pattern and process in plant ecology. Trends in Ecology and Evolution 16:529–530.

Niklas, K. J., J. J. Midgley, and B. J. Enquist. 2003. A general model for mass-growth-density relations across tree-dominated communities. Evolutionary Ecology Research 5:459–468.

Niu, H., R. Xu, and B. Song. 2000. Water Use Dynamic of *Leymus chinensis* Population. Acta Agrestia Sinica 8:226–232.
Pautasso, M., and P. J. Weisberg. 2008. Negative density-area relationship: the importance of the zeros. Global Ecology and Biogeography 17:203–210.

Ray, C., and A. Hastings. 1996. Density dependence: Are we searching at the wrong spatial scale? Journal of Animal Ecology 65:556–566.

Ripley, B. D. 1976. The second-order analysis of stationary point processes. Journal of Applied Probability 13:255–266.

Ripley, B. D. 1977. Modeling spatial patterns. Journal of the Royal Statistical Society B 39:172–192.

Ripley, B. D. 1981. Spatial statistics. Wiley, Hoboken, New Jersey, USA.

Root, R. B. 1973. Organisation of a plant-arthropod association in simple and diverse habitats: the fauna of collards (Brassica oleracea). Ecological Monographs 43:95–124.

Rothman, L. D., and D. C. Darling. 1990. Parasitoids of the goldenrod gall moth: effects of scale on spatial density dependence. Oecologia 83:1–6.

Schoener, T. W. 1986. Pattern in terrestrial vertebrate versus arthropod communities: Do systematic differences in regularity exist? Pages 556–586 in J. Diamond and T. J. Case, editors. Community ecology. Harper & Row, New York, New York, USA.

Smallwood, K. S., and C. Schonewald. 1996. Scaling population density and spatial pattern for terrestrial, mammalian carnivores. Oecologia 105:329–335.

Wang, W., C. Liang, Z. Liu, and D. Hao. 1999. Research on restoring succession of degenerated grassland in Inner Mongolia IV. Analysis of plant population dynamics during restoring succession. Journal of Arid Land Resources and Environment 13:44–55.

Wang, W., C. Liang, Z. Liu, and D. Hao. 2000a. Analysis of the plant individual behaviour during the degradation and restoring succession in steppe community. Acta Phytoecologica Sinica 24:268–274.

Wang, W., C. Liang, Z. Liu, and D. Hao. 2000b. Mechanism of degradation succession in Leymus chinensis and Stipa grandis steppe community. Acta Phytoecologica Sinica 24:468–472.

Wang, W., Z. Liu, D. Hao, and C. Liang. 1996a. Research on the restoring succession of the degenerated grassland in Inner Mongolia. I. Basic characteristics and driving force for restoration of the degenerated grassland. Acta Phytoecologica Sinica 20:449–459.

Wang, W., Z. Liu, D. Hao, and C. Liang. 1996b. Research on the restoring succession of the degenerated grassland in Inner Mongolia. II Analysis of the restoring processes. Acta Phytoecologica Sinica 20:460–471.

Wang, X., Y. Hou, F. Liu, Y. Chang, W. Wang, C. Liang, and B. Miao. 2011. Point pattern analysis of dominant populations in a degraded community in Leymus chinensis + Stipa grandis steppe in Inner Mongolia, China. Chinese Journal of Plant Ecology 35:1281–1289.

Wang, X., and C. Jiang. 2018. Spatial point pattern analysis in typical steppe under grazing disturbance. Science Press, Beijing, China.

Wang, X., C. Jiang, C. Jia, Y. Tai, Y. Hou, and W. Zhang. 2020. A new digital method of data collection for spatial point pattern analysis in grassland communities. Ecology and Evolution 10:7851–7860.

Wang, X., W. Wang, C. Liang, and Z. Liu. 2015. Using positive interaction ecology to explain grassland degradation induced by overgrazing. Chinese Science Bulletin 60:2794–2799.

Wang, Y., S. Yong, and Z. Liu. 1985. Vegetation of Inner Mongolia. First edition. Science Press, Beijing, China.

Westoby, M. 1984. The self-thinning rule. Advances in Ecological Research 14:167–225.

Wiegand, T., S. Gunatileke, and N. Gunatileke. 2007. Species association in a heterogeneous Sri Lankan dipterocarp forest. American Naturalist 170:77–95.

Wiegand, T., and K. A. Moloney. 2004. Ring, circles, and null-models for point pattern analysis in ecology. Oikos 104:209–229.

Wiegand, T., and K. A. Moloney. 2014. Handbook of spatial point pattern analysis in ecology. Chapman and Hall/CRC Press, Boca Raton, Florida, USA.

Yang, H., Y. Rong, and Z. Mu. 2016. Analysis of spatial heterogeneity of Leymus chinensis and Stipa grandis vegetation and soil nitrogen under different grazing intensities. Praticultural Science 10:1035–1043.

Yoda, K., T. Kira, H. Ogawa, and K. Hozumi. 1963. Self-thinning in overcrowded pure stands under cultivated and natural conditions. Journal of Biology of Osaka City University 14:107–129.

Zhu, Y., L. S. Comita, S. P. Hubbell, and K. P. Ma. 2015. Conspecific and phylogenetic density-dependent survival differs across life stages in a tropical forest. Journal of Ecology 103:957–966.

**Supporting Information**

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3275/full