The correlation analysis between ant mounds and plant resource in Olkhon region

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Abstract: Olkhon region in East Siberia has abundant and unique vegetation and animal resource for its peculiar geographic location, including ants. Ant, recognized as ecosystem engineers, has an important role in ecosystem. In order to investigate the ecological role of mound-building ants in this region, we focused our attention on the correlation between the distribution of ant mounds and plant species. Five quadrats (5 m × 5 m) were set up in this region, each of which was then divided into twenty-five quadrats (1 m × 1 m). We collected the location of every Black Bog Ant (Formica candida) mound, the number and biomass of various plants in every small quadrat. Using matrices, we tested the distribution pattern of ant mound randomly. The correlation between plants and ant mound pattern was tested by correlation analysis and regression analysis. The result showed that the spatial distribution of ant mound was random. We also found that Artemisia frigida, Carex duriuscula and Oxytropis sylvestris had a significant linear relationship with the spatial distribution of ant mound ($P < 0.05$), suggesting that the spatial distribution of ant mound was dependent on the spatial distribution of some plants. The underlying mechanism was further studied. We attributed this correlation to the feeding habits and foraging strategies of Black Bog Ant and tissue structure of these three plants. Our study figured out the interaction between Black Bog Ant and plant resource in Olkhon region, laying down the foundation for future study on the co-evolution of plant and animal resource in this unique ecosystem.

Keywords: ant mounds, spatial distribution pattern, plant biodiversity, correlation analysis, Olkhon region

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Корреляционный анализ между размещением муравейников и растительными ресурсами в Ольхонском регионе

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Резюме: Ольхонский регион как часть Восточной Сибири обладает богатыми и уникальными растительными и животными ресурсами благодаря специфическому географическому положению. Муравьи, в свою очередь, являются важной составной частью экосистемы и играют в ней важную роль. В целях исследования экологической роли муравьёв-строителей в этом регионе авторы данной статьи обратили внимание на корреляцию между расположением муравейников и видами растений. В районе исследований были выбраны пять пробных площадей (5 м × 5 м), каждая из которых затем была разделена на двадцать пять квадратов (1 м × 1 м). Отмечалось местонахождение каждого муравейника, сооруженного Formica candida, количество, видовой состав и биомасса различных растений в каждом малом квадрате. С использованием матриц случайным образом тестируется картина распределения муравейников. Корреляция между растениями и расположением насекомых проверялись с помощью корреляционного и регрессионного анализа. Результат показал, что пространственное распределение муравейников оказалось случайным. Также было обнаружено, что растения Artemisia frigida, Carex duriuscula и Oxytropis sylvestris имели значительную линейную связь с пространственным распределением муравейников ($P < 0.05$), предполагается, что пространственное распределение муравейников зависит от пространственного распределения некоторых растений. Авторы связали эту корреляцию с привычками питания и стратегиями старения черного болотного муравья, а также со структурой тканей указанных трех видов растений. Проведенное
Introduction

Olkhon region, located on the western shore of Lake Baikal in East Siberia, has a combined climate pattern of continental climate and maritime climate due to the huge water body of Lake Baikal. Its unique climate pattern and topography nurture rich biological resources, including some endemic species. A total of 118 families and 494 genera of vascular plants have been recorded in this area. Among them, Asteraceae, Poaceae, and Cyperaceae are the main families, and they account for 51% of genera and 59% of species. Animal resources are also abundant, with more than 1,600 aquatic animal species.

Among commonly spotted animals, ant, the highly socialized hymenopteran, is recognized as one of the most important arthropods in the soil who plays a vital role in ecosystem. The foraging behavior of ants plays an important role in the food web and energy cycle. It changes the flow of matter and energy - organic matter is buried deeper, small particles are brought to the surface, soil porosity is increased, moisture is dispersed and gas is released [1, 2]. The ant mounds surface is also rich in C, N and other elements [3]. These physical and chemical changes will affect the soil microenvironment and nutrient cycling, and then affect the structure of plant resources. Studies have proven that ants have formed a symbiotic relationship with many plants in a long-term evolution process [4]. For example, ants can help spread plant seeds and to some extent affect the community structure of some temperate forests [4]. On the other hand, plants provide food and habitat for ants. The reciprocal relationship between the ants and plants not only facilitates the restoration of natural communities, but also promotes their evolution. For these reasons, international studies have used ants as a biological indicator to detect environmental and biodiversity changes [5, 6]. However, the current research on ants mainly focuses on taxonomy, fauna and biodiversity [7]. Studies on other aspects, especially the correlation between their mound-building activities and plants are still very few.

Meanwhile, the spatial distribution pattern of insect populations is an important property of the population [8]. The spatial pattern of the population shows the distribution of individual in the horizontal space, which is the combinational result of the population characteristics, interspecies relationships, and environmental conditions [9]. It also reflects the population dynamics and community succession trend [10]. Therefore, the spatial distribution pattern of ant mounds is a good indicator of ant mound-building activity.

In this study, we investigated the spatial distribution pattern of Black Bog Ant (Formica candida), the dominant ant species in Olkhon region, and the distribution structure of plant resources. We set up a correlation model for the distribution of Black Bog Ant mounds and plants to explore their interaction.

Material and methods

The investigation was conducted on five 5 m × 5 m grassland at Chernorud Camp, Olkhon. Each quadrat was divided into 1 m × 1 m quadrats. Location of ant mounds, along with the quantities and biomass of plant species were recorded. Biomass was calculated based on the average weight of 3 individuals after air drying.

We used the $K$ function with the spatstat language pack of the statistic software R (3.5.2) to study the spatial distribution of the ant mounds [11, 12]. $K$ function was put forward by Ripley [13]:

$$K(r) = \frac{A}{n^2} \sum_{i=1}^{n} \sum_{j=1 \text{ and } j \neq i}^{n} w_{ij}^{-1} l(u_{ij}).$$
A represents the area of the quadrat, n represents the number of the ant mound in the quadrat, \( u_{ij} \) represents the distance between the ant mound \( i \) and \( j \), and \( w_{ij} \) represents weighting, which is used to eliminate the Edge Effect. Noticeably, when \( u_{ij} \leq r \), \( l(u_{ij}) = 1 \), and when \( u_{ij} > r \), \( l(u_{ij}) = 0 \).

We used Monte Carlo method with R (3.5.2) to generate the upper and lower envelop curve at random [14]. The number of the data point was the same with the observation point. If \( K(r) \) falls between two envelop curves, the ant mound resists to random distribution. If it is over the upper envelop curve, the ant mound resists to clumped distribution. If it falls below the lower envelop curve, the ant mound resists to uniform distribution [15].

Correlation analysis uses the Nonparametric tests to test significant difference between distributions of two population. In this experiment, the analysis was done by applying Spearman Analysis whose function is shown below [16].

\[
\rho = \frac{\sum_{i=1}^{n} (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n} (x_i - \bar{x})^2 \sum_{i=1}^{n} (y_i - \bar{y})^2}}
\]

Lm function with R 3.5.2 was applied for the full regression analysis. Full regression model of the total biomass of all collected plants and the ant mound was established. The variance inflation factor (VIF) was used to determine collinearity. We defined multicollinearity when VIF > 5. Stepwise regression was done by applying Step function for preliminary screening for variables and modeling with those candidates according to Akaike information criterion (AIC). Afterwards multiple regression model of the total biomass of the collected plants and the ant mound was established by the total biomass of each plant species. This model identified the plant species that have greater effect on the ant mound distribution in the overall environment and their corresponding effect.

**Results**

Figure 1 presents 188 *F. candida* mounds, including 20 from quadrat a, 17 from quadrat b, 40 from quadrat c, 35 from quadrat d and 76 from quadrat e.

According to the result of the K Function (Fig. 2), Kob values (the actual value of \( K(r) \)) of quadrat 1–3, 5 lie inside the envelope, so the ant mounds in these four quadrats are randomly distributed on the scale of 0–1.2 m. The result of quadrat 4 displays a different result. Ant mounds are clustered on the scale of 0–1 m, but resign to random distribution on a broader scale.

We identified 25 plant species at Chernorud Camp and yielded the biomass of each plants. Based on the biomass, we conducted correlation analysis by Spearman (Table 1). The results show that the biomass of *Artemisia frigida* (\( \rho = 0.442, P = 0.038 \)), *Carex duriuscula* (\( \rho_{0.10} = -0.367, P = 0.029 \)) and *Oxytropis sylvestris* (\( \rho_{19} = -0.287, P = 0.031 \)) have significant correlation with the distribution of ant mounds. The biomass of *Artemisia frigida* is positively correlated with ant mounds, while the biomass of the other two plants, *Carex duriuscula* and *Oxytropis sylvestris* has negative correlation individually. The result suggests extensive interaction between these three plants and ant mounds.

Shannon-Wiener biodiversity index and Simpson biodiversity index were selected as indicators of plant biodiversity to correlate with ant mounds on different scales. On the small scale (1 m × 1 m), the correlation coefficient of Shannon-Wiener biodiversity index (\( \rho_{S-W} \)) is 0.157 (\( P = 0.08 \)) and that of Simpson biodiversity index (\( \rho_{Simpson} \)) is 0.165 (\( P = 0.067 \)). Although the \( P \) values are all greater than 0.05, we still suppose that the spatial distribution of ant mounds and plant biodiversity tend to have a positive correlation.

When the scale comes to 5 m × 5 m, the \( P \) values are greater than 0.1 (Table. 2), suggesting that the spatial distribution of ant mounds has no correlation with plant biodiversity.

To exclude the influence of the possible correlations between variables, we further conducted the multiple regression analysis. We identified plant species that have multicollinearity, and chose the smallest model which contained the biomass of 8 plant species as independent variables by the stepwise regression (Table. 3). The output of the model after stepwise regression show that the distribution of ant mounds is positively correlated with the biomass of *O.spinosa*, *C.daurica*, *A.commutata* and *A.frigida*, while is negatively correlated with the biomass of *P.ambigua*, *C.squarrosa*, *O.sylvestris* and *C.duriuscula*.
Comparing the standard coefficients with each plants, *A. frigida* ($P = 0.031$), *O. sylvestris* ($P = 0.018$) and *C. duriuscula* ($P = 0.001$) have significant influence on the distribution of ant mounds. The result is consistent with that obtained from the correlation analysis.

The output of the model can be concluded as following:

$$Y = 1.829 + 0.019X_{O. spinosa} + 0.179X_{O. sylvestris} - 0.063X_{A. commutata} + 0.060X_{C. squarrosa} + 0.047X_{C. duriuscula} - 0.703X_{P. ambigua}$$

**Discussion**

In this study, by collecting the relevant data of plants and ant mound in Olkhon, we tried to
Ants, as the most widely distributed species and quantitative social insects on the earth, are socially ordered and efficient in foraging. To acquire the efficiency, ants adjust their foraging strategies and nesting positions to the resource distribution. Supposing the distribution of plants is greatly affected by environmental factors such as water and soil, the distribution of ant mounds in turn may be more affected by food resources. Thus, we hypothesized that the distribution of ant mounds is related to the distribution of plants species. In this survey, we found that plant biodiversity or most of the plant species had little impact on ant mound, with an almost no significant relationship. However, the total biomass of *Artemisia frigida*, *Carex duriuscula* and *Oxytropis sylvestris* had a more significant correlation with the numbers of nearby ant mounds.
Таблица 1. Корреляционный анализ между биомассой растений и распределением муравейников

| Plant ID | Scientific name                  | Plant biomass in each quadrat, g | Correlation coefficient ρ | P value |
|----------|----------------------------------|----------------------------------|---------------------------|---------|
| 1        | Agropyron cristatum              | 79.2, 2.4, 9.6, 36, 228.6        | 0.206                     | 0.304   |
| 2        | Allium ramosum                   | 0, 0, 0, 0, 37.1                 | 0.384                     | 0.86    |
| 3        | Allium schoenoprasum             | 0, 0, 0.9, 0, 0                | -0.014                    | 0.92    |
| 4        | Allium tenuissimum               | 0, 0, 0, 1.1, 0                | 0.008                     | 0.785   |
| 5        | Artemisia commutata              | 149.6, 58.3, 138.6, 80.3, 0    | -0.072                    | 0.066   |
| 6        | Artemisia frigida                | 0, 0, 0, 0, 433.6              | 0.442                     | 0.038   |
| 7        | Astér alpinus                    | 67.5, 192.5, 220, 247.5, 0     | -0.123                    | 0.967   |
| 8        | Bupleurum scorzonerifolium       | 121.8, 151.2, 56.7, 178.5, 3.2 | -0.206                    | 0.585   |
| 9        | Caragana pygmaea                 | 0, 0, 0, 17.4                  | 0.256                     | 0.377   |
| 10       | Carex duriuscula                 | 269.5, 21.7, 60.9, 128.8, 0    | -0.367                    | 0.029   |
| 11       | Carum carvi                      | 1.5, 9, 22.5, 15, 0            | 0.025                     | 0.669   |
| 12       | Cleistogenes squarrosa           | 0, 0, 0, 0, 15                 | 0.243                     | 0.162   |
| 13       | Cymbaria daurica                 | 0, 0, 0, 69.6                  | 0.382                     | 0.108   |
| 14       | Eremogone meyeri                 | 379.5, 21, 432, 175.5, 152     | 0.074                     | 0.902   |
| 15       | Filifolium sibiricum             | 46.5, 1.1, 15, 3, 0            | -0.248                    | 0.733   |
| 16       | Iris ruthenica                   | 2.4, 0.6, 1.8, 3.6, 0          | -0.085                    | 0.733   |
| 17       | Koeleria macrantha               | 0, 0, 0, 10                    | 0.402                     | 0.731   |
| 18       | Orostachys spinosa               | 208.8, 72, 180, 122.4, 2.8     | -0.003                    | 0.256   |
| 19       | Oxytropis sylvestris             | 190.9, 243.8, 197.8, 46, 0     | -0.287                    | 0.031   |
| 20       | Potentilla biturca               | 0, 0, 0, 31.5                  | 0.265                     | 0.94    |
| 21       | Potentilla tanacetifolia         | 2.2, 2, 0, 1.1                  | -0.08                     | 0.846   |
| 22       | Ptilotrichum sibiricum           | 11.9, 0.3, 0.1, 6.4, 5.8       | 0.146                     | 0.543   |
| 23       | Pulsatilla ambigua               | 130.5, 109.5, 124.5, 85.5, 0   | -0.281                    | 0.219   |
| 24       | Sanguisorba officinalis          | 70.3, 3.7, 85.1, 0, 0          | -0.075                    | 0.448   |
| 25       | Thymus serpillum                 | 33, 78.1, 85.8, 23.1, 0        | -0.146                    | 0.583   |

Таблица 2. Корреляционный анализ между индексом биоразнообразия растений и муравейниками в масштабе 5 м х 5 м

| Plant biodiversity | Quadrat number | Correlation coefficient | P value |
|--------------------|----------------|-------------------------|---------|
|                    | 1              | 2                       | 3       | 4       | 5       |
| Shannon-Wiener     | 2.162          | 2.207                   | 2.174   | 2.271   | 1.973   | -0.8    | 0.107   |
| Simpson            | 0.841          | 0.87                    | 0.847   | 0.871   | 0.825   | -0.68   | 0.21    |

Таблица 3. Коэффициенты регрессионной модели ($R^2 = 0.358$)

| Modeling           | Regression coefficients | Standard deviation | Standard coefficient | T value | P value |
|--------------------|-------------------------|--------------------|----------------------|---------|---------|
| Constant           | 1.829                   | 0.306              | −                    | 5.984   | 0       |
| C.spinosa          | 0.019                   | 0.013              | 0.114                | 1.481   | 0.141   |
| C.daurica          | 0.179                   | 0.115              | 0.219                | 1.563   | 0.121   |
| P.ambigua          | -0.063                  | 0.039              | -0.125               | -1.605  | 0.111   |
| C.squarrosa        | -0.703                  | 0.386              | -0.154               | -1.822  | 0.071   |
| A.communita        | 0.047                   | 0.025              | 0.174                | 1.906   | 0.059   |
| A.frigida          | 0.06                    | 0.027              | 0.392                | 2.179   | 0.031   |
| O.sylvestris       | -0.062                  | 0.026              | -0.238               | -2.396  | 0.018   |
| C.duriuscula       | -0.063                  | 0.018              | -0.297               | -3.403  | 0.001   |
Among them, the biomass of *Artemisia frigida* was positively related to the number of ant mounds. We suggested three possible reasons. First, the special properties of *Artemisia frigida* make it more attractive to more insects [17], providing sufficient food source for the bright black ant. Second, the root structure of *Artemisia frigida* stabilizes the rhizosphere soil ecosystem [18] which may be suitable for ants' living. Third, there may be a mutually beneficial relationship between *Artemisia frigida* and the bright black ant. The seed surface of some plants is covered with a nutritional part that attracts ants – oleosome [4]. *Artemisia frigida* may also provide nutrition for ants through other specific structures. Ants will in return protect the plants in the way of preventing or reducing from feeding and destruction of plants by other herbivores except honeydew insects. Such properties of *Artemisia frigida* may explain for the fact that the number of ant mounds was positively correlated with the total biomass of *Artemisia frigida* [19].

In addition, the individual total biomass of *Carex duriuscula* and *Oxytropis sylvestris* was negatively related to the number of ant mounds nearby. This phenomenon could be explained by the unfavorable properties of these two plants in forming a reciprocal symbiosis relationship with the bright black ant. According to the existing research, the bright black ant has reached nutritional symbiosis with Homoptera. The ants collect nectar from Homoptera, which is the nutrients excreted by Homoptera [20]. However, the covering villi of *Oxytropis sylvestris* which help resist insects and low temperature can reduce palatability [21]. Meanwhile *Oxytropis sylvestris* contains a variety of toxic alkaloids [22], making it not attractive to Homoptera insects. Therefore, this could be the reason for the finding that the nest distribution of bright black ants was individually negatively related to these two plants.

**Conclusion**

There is little research on the relationship between the spatial pattern of ant mound and plants. Thus, our research provides a different research idea for the interaction of animals and plants. Future research can increase the amount or coverage of quadrats or cover more plant species. Considering scale effect [23], it is necessary to investigate the spatial pattern distribution of ant nest on different scales, given this study is only based on the scale of 5 m × 5 m. Given that more information is obtained and considered, we could make one step further to build a bond between biological resources and tectonic motion, thus investigating how the unique ecosystem Baikal Lake evolved.

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