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Comparison of ant community composition among three urban habitats: a case study in Nanchong, Sichuan

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Abstract: As an important human-caused disturbance factor, urbanization has significantly changed the structure of natural habitats and influenced the composition of animal communities. The characterizations of abundant population amount, high species diversity, and easy to be collected of ants make these species become excellent models for studying the effects of land-use types on the composition of animal communities in urban areas. In May 2018, ants were sampled from 3 land-use types ('garden and Greenland', 'dry farm' and 'woodland') using pitfall traps in an urban area of Nanchong city, Sichuan province. A total of 1 847 ant individuals were collected. The proportion of *tetramorium tsushimae* was the highest in 'garden and Greenland' (73.80%), and significantly higher than that of *monomorium chinense* in 'dry farm' (25.48%) and *Pheidole nodus* in 'woodland' (28.79%). There was no significant difference in the number of individuals and species richness among the 3 habitats, but the Simpson diversity index and Pielou evenness index of ant community in the habitat of 'garden and Greenland' were significantly lower than those in the habitats of 'dry farm' and 'woodland'; the composition of ant community in 'woodland' was moderately different from that in 'garden and Greenland' ($q = 0.444$) and 'dry farm' ($q = 0.500$), and the community composition in 'garden and Greenland' and 'dry farm' ($q = 0.647$) was moderately similar. Therefore, the compositions of ant communities are varied in different land-use types in urban areas, and the habitat of 'garden and Greenland' is more likely to facilitate the formation of dominant species than 'dry farm' and 'woodland'.

Keywords: community ecology; biodiversity; urbanization; garden and Greenland; woodland; dry farm

1. Introduction

The process of global urbanization has accelerated significantly since the 20th century (Grimm et al., 2008). Urban expansion has transformed natural habitats into various forms of artificial landscapes, and changed local environmental conditions such as climate and soil properties (Pataki et al., 2006; Rocha Ortega & Castaño-Meneses, 2015). These changes may reduce the migration capacity of terrestrial animals, affect the resource flow in natural patches, and lead to the rapid loss of local biodiversity (Lessard & Buddle, 2005; Hahs et al., 2009; Alberti, 2015). However, urbanization has also created suitable habitats for some species that can adapt to urban habitats (Buczowski & Richmond, 2012), increasing genetic, taxonomic or functional similarities among communities, and thus promoting biological homogenization (McKinney & Lockwood, 2001; Olden et al., 2004; Newbold et al., 2015).

Ants (Hymenoptera Formicidae) are important groups indicating biodiversity and environmental change (King et al., 1998; Hoffmann, 2010; Li Qiao et al., 2011; Liu

Xia et al., 2017). First of all, ants provide important ecosystem services, such as predators, mutual benefits and prey in food webs (Heil and McKey, 2003). Secondly, ants are widely distributed, with a large number of species and numbers, which has a relatively short generation interval and is conducive to sample collection (Guo et al., 2014; Li Qiao et al., 2015). In addition, the vast majority of ant species have weak migration ability, are easy to be hindered by natural geographical barriers, and are particularly sensitive to changes in the microenvironment, and can respond quickly to them (McKinney, 2008; Stringer et al., 2009).

In this study, three types of land use, namely “parks and greenbelts”, “drylands” and “arbor forests”, were selected in the urban area of Nanchong City, Sichuan Province, to investigate and compare the composition of ant communities, which will not only provide basic data for studying the impact of urbanization on the structure of wildlife communities, but also test the effectiveness of ant communities as biological indicator species to reflect the response of the ecosystem.

2. Materials and methods

2.1. Research location

Nanchong City is located in the northeast of Sichuan Basin and the middle and lower reaches of Jialing River. It is located in the subtropical monsoon climate zone with abundant water and heat conditions in the southwest of China, and in the transitional zone from the Oriental to the Palearctic in terms of the continental animal geographical division (Zhang and Zheng, 2002). Influenced by the climate of the basin, there are four distinct seasons, with the same period of rain and heat. The annual average precipitation is 980~1150 mm; the average annual temperature is 15.8~17.8 °C; Relative humidity 76%~86% (Xu et al., 2015); the annual average sunshine duration is 1200~1500 h. According to the existing survey, there are 78 species of ants distributed in Sichuan Province (including Chongqing) (Zhang and Zheng, 2002) and 26 species in urban habitats (Tan et al., 2010).

2.2. Research methods

2.2.1. Habitat vegetation survey and ant collection method

With reference to the secondary classification in the Classification Standards for Land Use Status (GB/T21010–2017) approved and issued by the AQSIQ and the National Standardization Administration, three land use types, namely “Park and Green Land” (code 0810), “Dry Land” (code 0103) and “Arbor Forest Land” (code 0301), are selected in Huafeng Campus of West China Normal University in Nanchong City, and eight 1m are set respectively \times 1 m quadrat. With reference to the method of McIntyre et al. (2001), the habitat vegetation survey was conducted in the area with the center point of the quadrat as the center of the circle and a radius of 20 m. Habitat survey indicators include herbaceous vegetation coverage, forest canopy density and Shannon Wiener diversity index (H) of vegetation. According to the spot measurement method, a sharp needle shall be vertically placed every 10 cm along the diagonal of the quadrat. The diameter of each needle shall not exceed 2 mm. For vegetation less than 2 m, the number of branches and leaves hit by the needle shall be recorded. The

ratio of this number to the total number of needles is the coverage of herbaceous vegetation (Heraldol et al., 2010). The stand canopy density is the ratio of the number of needles vertically covered by the crown to the total number of needles (Magnusson et al., 1999). In order to calculate the Shannon Wiener diversity index of vegetation, five sub sample circles with a radius of 3 m were set in the sample circle with a radius of 20 m (the center spacing was not less than 10 m), and the species and quantity of arbors were investigated (Song and Gao, 2002; Cao, 2006; Wang, 2011; Si et al., 2019); And set five 1 m × 1 m sub sample, investigate the species and quantity of herbs (Wang, 2011).

There were significant differences in herbaceous vegetation coverage ($F = 5.68$, $df = 2$, $P < 0.05$), stand canopy density ($F = 1.86$, $df = 2$, $P < 0.05$) and Shannon Wiener diversity index ($F = 8.79$, $df = 2$, $P < 0.05$) among the three habitats. There are many kinds of trees (*Robinia pseudoacacia*, *Koelreuteria paniculata*, *Bischofia polycarpa*, *Ilex chinensis*, etc.) and herbs in the “arbor forest land” (*Imperata cylindrica*, *Taraxacum mongolicum*, *Portulaca oleracea*, *Leonurus japonicus*, *Carpesium acrotanoids*, *Pteris cretica*, *Ophioglossum vulgatum*, etc.), almost no shrubs are distributed. The main crop of “dry land” in May is maize (*zea mays*), with scattered distribution of *pterocarya stenoptera* and *cinnamomum camphora*, and few other herbs except crops. The arbors in the “parks and green spaces” are mainly *cinnamomum camphora*, while the herbaceous vegetation is mainly the terrace grass *ophiopon bodinieri*. *Cynodon dactylon*, *trifolium repens*, *digitaria sanguinalis*, *Malan (aster indicus)* are scattered. Therefore, these three land use types have different vegetation conditions and represent different habitat types.

In May 2018, ant samples were collected from the above 24 quadrats by trap method. The collection duration is 48h. The spacing between quadrats in different habitats shall not be less than 1km, and the spacing between quadrats in the same habitat shall not be less than 10m. Traps are set in the flat area at the top corner of the quadrat. The trap consists of two 500 mL plastic cup stacks, and the inner cup lip is flush with the ground to avoid cup damage during continuous sampling (McIntyre et al., 2001). Pour 150 mL propylene glycol into the plastic cup to kill ants and effectively preserve them in a short time. The collected samples were stored in 70% ethanol solution and stored in the specimen bank of West China Normal University.

2.2.2. Classification and identification of ants

The collected ant samples were sequenced using DNA barcode technology, and the CO I sequencing results were compared with those of GenBank (<http://www.ncbi.nlm.nih.gov/>) and BOLD Systems (<http://www.boldsystems.org/>). The sequence is compared. This result is used to quickly determine the phylogenetic lineage to which it belongs, so as to facilitate the subsequent morphological comparison. In the process of DNA extraction, the abdomen of the ant is first removed, and then the remaining limbs are placed in a mortar and cut into pieces with surgical scissors; Joining 500 μ L DNA extract and 20 μ L 10 mg/mL protease K, water bath at 57 °C for 2–3 h; adding equal volume of balanced phenol, mix well, and take the supernatant after centrifugation. We add equal volume of chloroform isoamyl alcohol mixed solution, and take the supernatant after centrifugation. We add 2 volumes of pre cooled absolute ethanol and 1/10 volume of sodium acetate to retain the sediment. We add

1mL 70% ethanol solution into the sediment, and discard the supernatant after centrifugation (Li and Xu, 2010; Chen, 2014); Place the centrifuge tube open until the ethanol is completely volatilized, and add 40 μ L ddH₂O, stored at 20 °C for standby (Chen, 2014). The CO I fragment was amplified with universal primers LCOI490 and HCO₂198 (Folmer et al., 1994; Chen, 2014). DNA extraction and sequencing were completed by the Southern Center of Life Barcode, Kunming Institute of Zoology, Chinese Academy of Sciences.

For species morphology identification, refer to “A new general catalog of the ants of the world” (Bolton, 1995), “Chinese Economic Insecta Vol. 47 Hymenoptera Formicidae (I)” (Tang et al., 1995), “Chinese Ants” (Wu and Wang, 1995), “Guangxi Ants” (Zhou, 2001), “Research on the Classification of Ants in Hubei Province” (Wang et al., 2009) and other monographs, as well as “Encyclopedia of Life”(http://eol.org/)Ant Net(http://www.ants-china.com/?sp=6604), “ANTWEB”(http://www.antweb.org/), “Ant Wiki”(http://www.antwiki.org/wiki/Welcome_to_AntWiki) Etc. The morphological characteristics of ants were observed with LEICAm²05C stereomicroscope.

Table 1. Species and individual numbers of ants sampled from 3 habitat types in Nanchong, Sichuan.

species	Number of individuals/piece		
	Arbor woodland	dry land	Parks and green spaces
1. <i>Dolichoderinae</i>			
a. <i>Ochetellus glaber</i>	4	—	—
2. <i>Formicinae</i>			
b. <i>Camponotus japonicus</i>	3	5	1
c. <i>Nylanderia bourbonica</i>	5	70	3
d. <i>Nylanderia/lavipes</i>	11	—	20
e. <i>Plagiolepis manczshurica</i>	16	1	2
3. <i>Myrmicinae</i>			
f. <i>Aphaenogaster japonica</i>	—	75	1
g. <i>Crematogaster vagula</i>	2	—	—
h. <i>Crematogaster rogenhoferi</i>	—	—	19
j. <i>Myrmecina americana</i>	—	—	9
k. <i>Monomorium pharaonis</i>	6	37	12
l. <i>Monomorium chinense</i>	71	198	109
m. <i>Pheidole mendanai</i>	4	—	7
n. <i>Pheidole nodus</i>	74	175	10
o. <i>Strumigenys/ormosa</i>	—	—	1
p. <i>Tetramorium shensiense</i>	10	36	11
q. <i>Tetramorium tonganum</i>	—	20	—
r. <i>Tetramorium tsushimae</i>	36	157	600
4. <i>Ponerinae</i>			
s. <i>Leptogenys chinensis</i>	7	—	—
t. <i>Odontomachus monticola</i>	8	—	—

2.2.3. Data processing

The species number (S), Shannon Wiener diversity index (H), Simpson diversity index (D) and Pielou evenness index (J) were used to reflect the species richness, species diversity, species dominance and species evenness of ant communities in each quadrat, and the least significant difference in One Way ANOVA was used to evaluate the differences of each index of ant communities among the three habitats (the significance level was set as $\alpha = 0.05$). $H = -\sum P_i \ln P_i$ (Li et al., 2018); $D = 1 - \sum_{i=1}^S (P_i)^2 = 1 - \sum_{i=1}^S (N_i/N)^2$, P_i is the proportion of the number of individuals of species i to the total number of individuals (Sun and Zheng, 2012); $J = H/\ln S$ (Li et al., 2018). Continuous variables are represented by $\bar{x} \pm SE$. The jaccard similarity index was used to compare the similarity of ant community composition in three habitats, $q = c/(a + b - c)$, where c is the number of common species in two communities, and a and b are the number of species in community A and community B respectively (Mei et al., 2006). According to the Jaccard similarity principle, when q is 0~0.250, it is very different; when q is 0.251~0.500, it is moderately different; when q is 0.501~0.750, it is moderately similar; when q is 0.751~1.000, it is very similar (Yang et al., 2014).

3. Results

3.1. Type and quantity

In this study, 1847 ants were collected. The CO I gene (204 bp) of 18 ants was detected, and the sequence similarity between 6 ants and GenBank and BOLD Systems reached 98%~99% (*Tetramorium tsushimae*: AY641700; *Monomorium chinense*: MH754300; *Pheidole nodus*: EF518375; *Nylanderia bourbonica*: EF609962; *Ochetellus glaber*: JQ913605; *Nylanderia flavipes*: MH754327); The similarity between the 12 ant species and the sequence is 84%~96% (*Monomorium pharaonis*: KJ861846; *Plagiolepis manczshurica*: AB019423; *Camponotus japonicus*: KR784733; *Leptogenys chinensis*: KC685034; *Odontomachus monticola*: FX986057; *Myrmecina americana*: KR421703; *Aphaenogaster japonica*: JQ742634; *Pheidole mendani*: KJ142033; *Crematogaster vagula*: MH754499; *Crematogaster rogenhoferi*: EU605790; *Tetramorium tonganum*: MG567163; *Tetramorium lanuginosum*: GU709681). According to morphological comparison, 5 individuals are of the same species, which can only be identified as Formicidae sp. of Formicidae sp., and 6 individuals of the same species can only be identified as *tetramorium* sp; The remaining 1836 individuals belong to 4 subfamilies, 13 genera and 19 species (**Table 1**), including 1680 *myrmicinae* specimens belonging to 7 genera, 12 species, 137 *formicinae* specimens belonging to 3 genera, 4 species, 3 genera, 15 *ponerinae* specimens belonging to 2 genera, 2 species, and 4 *dolichoderinae* specimens belonging to 1 genus and 1 species. There are 8 kinds of ants distributed in the 3 habitats (Tsujiima pavement ant, Chinese small house ant, broad knot big head ant, Bruni's nest ant, Shaanxi pavement ant, *Tetramorium shensiense*, Pharaoh small house ant, Manchuria oblique knot ant and Japanese bow-back ant), and 4 kinds of ants are only found in the "arbor forest" (Chinese fine jawed ant, mountain big tooth ant, hairless pit stink ant and floating belly ant), The 3 species

of ants are only found in “parks and green spaces” (*Strumigenys formosa*, *Formosan taurus*, American leaf cutter), and Tonga road ants are only found in “dry lands”. However, the samples with 84% similarity to the COI gene sequence of *P. velveticus* belong to the same genus of *P. pilaris* in Shaanxi.

813 ants (101.62 ± 25.18 , $n = 8$) were collected in “Park and Green Space”, of which 600 ants (75.00 ± 26.52 , $n = 8$) were collected in “Park and Green Space”, accounting for 73.80% ($66\% \pm 10\%$, $n = 8$) of the total number of ants in “Park and Green Space”; 257 specimens (32.13 ± 12.14 , $n = 8$) were collected in the “arbor forest land”, of which 74 (9.25 ± 6.31 , $n = 8$) were the largest, accounting for 28.79% ($18\% \pm 6\%$, $n = 8$) of the total specimens in the “arbor forest land”; A total of 777 specimens (97.13 ± 34.79 , $n = 8$) were collected in “dry land”, of which 198 (24.75 ± 11.70 , $n = 8$) were the largest, accounting for 25.48% ($23\% \pm 6\%$, $n = 8$) of the total number of “dry land” specimens. The proportion of individuals in the ant community of “parks and green spaces” was significantly higher than that in the ant community of “arbor forest land” ($F = 11.651$, $df = 2$, $P < 0.05$).

3.2. Biodiversity comparison

There was no significant difference in the individual number ($F = 2.303$, $df = 2$, $P > 0.05$), species number ($F = 0.078$, $df = 2$, $P > 0.05$) and Shannon Wiener diversity index ($F = 6.651$, $df = 2$, $P > 0.05$) of ant communities among the three habitats; Simpson diversity index ($F = 8.902$, $df = 2$, $P < 0.05$) and Pielou evenness index ($F = 14.211$, $df = 2$, $P < 0.05$) are “arbor forest land” > “dry land” > “park and green land” (Table 2). The similarity coefficient between “dry land” and “arbor forest land” is 0.500, while that between “park and green land” and “arbor forest land” is 0.444, which is medium and different; The similarity coefficient of “park and green land” and “dry land” is 0.647, which is moderately similar.

Table 2. Biodiversity index of ants sampled from 3 habitat types in Nanchong, Sichuan.

Habitat type	Number of species	Shannon-Wiener	Simpson	Pielou
Arbor woodland	$6.38 \pm 0.89a$	$1.46 \pm 0.08a$	$0.70 \pm 0.02a$	$0.83 \pm 0.03a$
dry land	$6.25 \pm 0.90a$	$1.39 \pm 0.13ab$	$0.69 \pm 0.04a$	$0.81 \pm 0.04a$
Parks and green spaces	$5.88 \pm 1.01a$	$0.80 \pm 0.19b$	$0.39 \pm 0.09b$	$0.45 \pm 0.09b$

Notes: different superscripts indicate there is a significant difference between the diversity indexes of ant communities in different habitats.

4. Discussion

In the urban ecosystem, the development and utilization of land mainly serve for human life. Therefore, the vegetation and topsoil of its original habitat have been removed, making it different from the undeveloped natural habitat and unique. The results showed that there were significant differences in ant community composition among the three urban habitats of “park and green land”, “arbor forest land” and “dry land”. Among them, Tsujima pavement ant is more adaptable than other species in “parks and green spaces”, and its individual number has obvious advantages in the community.

The collection results of ant species and individuals will be affected by the collection methods. Trapping is a simple and cheap collection method (Huang et al., 2013), which has high efficiency in open habitats (Melbourne, 1999) and little damage to habitats. This method can effectively collect species with strong motor ability, wide range of motion and nocturnal activities (Sabua and Shiju, 2010). Although some people once expressed concern that the trap method could not effectively collect species with small or slow activity space (Spence and Niemel, 1994). However, relevant research shows that the trap method is still one of the most ideal methods for collecting ants, and is widely used in biodiversity monitoring (Work et al., 2002; Sabua and Shiju, 2010).

The structural differences between different habitats in the city will affect the species richness and biodiversity of animal communities (Pacheco and Vassconcelos, 2012; Pardee and Philpott, 2014; Mata et al., 2017). In particular, good vegetation conditions (vegetation species diversity and spatial heterogeneity, etc.) can provide more abundant and high-quality nesting sites, hiding places and food resources for more ant species (Mong et al., 2004; Buczkowski and Richmond, 2012). Compared with “parks and green lands” and “dry lands”, “arbor forest land” has higher woody plant richness and stand canopy density, and more branches and leaves are covered on the ground, which may help more ant species coexist in it. In “park and green land” and “dry land”, the species of herbaceous vegetation is relatively single, and the similarity of vegetation characteristics is high. During this period, the widespread use of pesticides may also lead to the disappearance of some ant species or the reduction of the number of individuals (Brittain et al., 2010; Sonoda et al., 2011). In addition, conventional farming behavior and soil disturbance may also be important reasons for the decrease of biodiversity of ant communities in “dry land” (Altieri, 1999; Uno et al., 2010). In this study, the impact of habitat types on ant communities was not reflected in the absolute number of species and individuals, but the ant diversity index of “parks and green spaces” and “drylands” was lower than that of “arbor forests”, and there were also obvious dominant species in the “parks and green spaces” habitats.

Tsujima pavement ant is an omnivorous and aggressive species with strong competitiveness. In the “parks and green spaces” near human settlements, Tsushima pavement ants often have behavioral and quantitative advantages, and may affect the diversity of local ant communities through the interaction of competition or exploitation (Lessard and Buddle, 2005). At present, there is still a lack of ecological data on Tsushima Pavillion Ant, and its biological data, competition mechanism with other native ant species and ecological consequences of becoming a dominant species need to be further studied.

The results of this study confirmed that the ant community can respond well to different urban habitat types. In the future research, the indicator function of ant community can be fully used to reflect the impact of land use types and landscape patterns on ecosystem functions. In the process of urbanization, while meeting human needs, we should also plan land use more reasonably from the perspective of maintaining biodiversity to build a more livable urban ecosystem for people and associated animals. Therefore, it is necessary to establish a long-term and more extensive monitoring and research system, involving more cities, habitat types and animal groups, and to better

understand the impact of urbanization on biodiversity.

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