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Effects of microhabitat on rodent-mediated seed removal of endangered *Kmeria septentrionalis* in the karst habitat, Guangxi, southwestern China

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Ground-dwelling rodents play an important role in the dynamics of forest plant seeds that fall to the ground. Studies have established that rodent-mediated seed removal is affected by different microhabitats; however, the ways in which rodents remove fallen seeds distributed in microhabitats within karst ecosystems remains unclear. This study investigated *Kmeria septentrionalis* seed removal by rodents in four microhabitats (stone cavern, stone groove, stone surface, and soil surface) in karst with three types of seeds (fresh seeds, black seeds (intact seeds with black aril that dehydrates and darkens), and exposed seeds (clean seeds without aril) to clarify the effect of microhabitat on rodent-mediated seed removal. *Rattus norvegicus*, *Leopoldamys edwardsi*, and *R. flavipectus* were the rodents that mainly consumed and removed the seeds. There was a high removal rate for all seed types in the four microhabitats, and there were significant differences in seed removal rates among different microhabitats and seed types. Rodents have a tendency to removing seeds from the three stone related microhabitats (highest in stone caves, 69.71±2.74%) than those from the soil surface (lowest, 53.90±2.92%), and the exposed seeds were more attractive to rodents (76.25±2.20%) than fresh seeds (36.18±2.29%). We deduced that seeds falling on the soil surface would incur a lower predation risk and that there would be better survival possibilities for the germinated seeds. Therefore, our results indicate that when seeds are dropped or removed to the soil surface by birds, the lower predation rate of rodents will increase the survival of this endangered species.

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ABSTRACT

Ground-dwelling rodents play an important role in the dynamics of forest plant seeds that fall to the ground. Studies have established that rodent-mediated seed removal is affected by different microhabitats; however, the ways in which rodents remove fallen seeds distributed in microhabitats within karst ecosystems remains unclear. This study investigated *Kmeria septentrionalis* seed removal by rodents in four microhabitats (stone cavern, stone groove, stone surface, and soil surface) in karst with three types of seeds (fresh seeds, black seeds (intact seeds with black aril that dehydrates and darkens), and exposed seeds (clean seeds without aril) to clarify the effect of microhabitat on rodent-mediated seed removal. *Rattus norvegicus*, *Leopoldamys edwardsi*, and *R. flavipectus* were the rodents that mainly consumed and removed the seeds. There was a high removal rate for all seed types in the four microhabitats, and there were significant differences in seed removal rates among different microhabitats and seed types. Rodents have a tendency to removing seeds from the three stone related microhabitats (highest in stone caves, 69.71±2.74%) than those from the soil surface (lowest, 53.90±2.92%), and the exposed seeds were more attractive to rodents (76.25±2.20%) than fresh seeds (36.18±2.29%). We deduced that seeds falling on the soil surface would incur a lower predation risk and that there would be better survival possibilities for the germinated seeds. Therefore, our results indicate that when seeds are dropped or removed to the soil surface by birds, the lower predation rate of rodents will increase the survival of this endangered species.

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Keywords: *Kmeria septentrionalis*; Seed removal; Microhabitat; Seed type; Karst habitat

INTRODUCTION

Seed removal by rodents is one of the most important dispersal mechanisms for many plant species (*Lichti et al., 2017*; *Wang & Corlett*, *2017*). The seed-hoarding behavior of rodents not only helps transport seeds away from the mother tree, where they are likely to suffer strong parental competition (*Nathan & Muller-Landau*, *2000*; *Jansen et al*., *2014*), but also increases the chances of seeds reaching a suitable germination habitat, thus facilitating the expansion of their distribution (*Steele et al*., *2015*; *Wang & Corlett*, *2017*). However, the pattern and intensity of seed removal depend on a multitude of biotic and abiotic factors, such as seed traits and availability (*García et al*., *2011*; *Xiao et al*., *2015*), rodent abundance (*Li & Zhang*, *2007*) and habitat characteristics (*Wang et al*., *2019*; *Perea et al*., *2012*). Of all the parameters, the microhabitat where the seeds are located has been one of the most important factors affecting seed removal (*García-Castaño et al*., *2006*; *Steele et al*., *2015*).

Microhabitats in which the seeds are located not only provide a variety of biological and abiotic conditions for the survival of rodents (*Vander-wall*, *2000*; *Fleury & Galetti*, *2006*), but also represent the quality of the foraging habitat (e.g., predation risk and foraging cost). These factors could change rodent abundance, activity intensity, and spatial distribution (*Pérez-Ramos & Marañón*, *2008*), ultimately affecting the probability of seed encounter and the foraging behavior decisions (e.g., removal or in situ consumption) by rodents in relation to seeds (*Perea et al*., *2012*; *Reed et al*., *2005*). For instance, *Peromyscus polionotus* removed more seeds from dense vegetation cover than from open patches where the possibility of being confronted by predators was high (*Orrock et al*., *2011*). The foraging behavior of rodents with respect to seeds in different microhabitats affects seedling regeneration, spatial distribution and diversity of trees (*Hirsch et al*., *2012*; *Zhang et al*., *2016*). For instance, Steele et al. (2014) found that eastern gray squirrels (*Sciurus carolinensis*) dispersed larger, more profitable acorns of oaks (*Quercus spp*.) into open habitats, where the cache pilferage was lower, and conditions were advantageous to seedling establishment.

Rodent-mediated seed removal is also affected by changes in seed morphology (e.g., seed size, chemical content, energy/nutrient content), and some studies have shown that rodents prefer to remove and cache larger seeds with higher nutritional value as food reserves (*Rusch et al*., *2013*; *Xiao et al*., *2015*). In addition, it appears that the changes in seed morphology after treatment by primary dispersers also influence removal by rodents (*Perea et al*., *2011*; *Pan et al*., *2016*). With regard to seed removal, rodents seem to prefer exposed seeds regurgitated or defecated by birds to intact seeds (*Perea et al*., *2011*). However, these studies have involved different plants, and whether seeds of different morphology from the same plant will affect the removal by rodent is still unclear.

Kmeria septentrionalis is a dioecious tree species of the Magnoliaceae endemic to China, has been listed as an endangered (EN) species by the IUCN, and is a first-class national protected plant in China (*Lin et al*., *2011*). The tree seeds have red aril and rely on birds for dispersal. After passing through the digestive tract of birds, the seeds become completely exposed (i.e., have no pulp) (*Wang et al*., *2019*). However, seeds not removed by birds fall to the ground and remain intact (fresh seeds), and the red aril of fresh seeds becomes dehydrated and turn black after falling on the ground for about 138.20 ± 3.86 h (n=30) (black seeds). Moreover, due to the high topographic heterogeneity of karst habitat, several types of microhabitats (e.g., stone groove, stone crevice, and stone cavern) tend to form (*Clements et al*., *2006*; *Zhang et al*., *2013*). Therefore, these three types of seeds (fresh seeds, black seeds, and exposed seeds) are often randomly distributed in different microhabitats under the mother trees.

In this study, we performed a series of rodent-mediated seed removal experiments in four different karst microhabitats (stone cavern, stone groove, stone surface, and soil surface) with three types of *K. septentrionalis* seeds (fresh seeds, black seeds, and exposed seeds) to determine how karst microhabitat affects the seed removal rate by rodents. We addressed the following questions: (1) How do the microhabitats in karst habitat affect the seed removal of *K. septentrionalis* by rodents? (2) Do rodents have preferences for a specific type of seeds?

MATERIALS & METHODS

Study area

Field work was performed in the Mulun National Nature Reserve (107°54′01″-108°05′51″E; 25°07′01″-25°12′22″N) in Guangxi Zhuang Autonomous Region, southwest of China (Fig.1). The nature reserve has typical karst landforms, mainly consisting of peak-cluster depressions and valleys, with altitudes ranging from 300-1000 m above sea level, and a total area of 10829.7 hm^2. The annual average temperature recorded was 19.3 °C; the extreme minimum temperature was -5 °C in January, and the maximum temperature was 26.7 °C in July. The reserve belongs to the mid-subtropical monsoon climatic zone and has an annual average precipitation of 1529.2 mm (*Liu et al*., *2012*). Rainfall occurs mostly between June and September, the annual total temperature recorded was 4700-6300 ℃, the annual frost-free period lasted for 235-290 days and the relative humidity was more than 79% (*Pan et al*., *2008*). The local vegetation was middle subtropical evergreen and deciduous broad-leaved mixed forest dominated by species such as *Kmeria septentrionalis*, *Lindera communis*, *Machilus pingii*, and *Loropetalum chinense* (*Wang et al*., *2019*).

Study species

Kmeria septentrionalis has been listed as an endangered (EN) species by the IUCN (*Lin et al*., *2011*) in China. It is found only distribution in the karst habitat in Guangxi Zhuang Autonomous Region (Luocheng, Huanjiang), Guizhou Province (Libo) and Yunnan Province (Malipo, Maguan), and the largest clumped distribution has been reported in Mulun National Nature Reserve, where the mother trees more than 200 (*Pan et al*., *2008*). Every year, female plants bear 100-300 fruits, and each fruit contains 4-14 seeds (mean ± standard error, length, 1.14 ± 0.15 cm; width, 0.49 ± 0.06 cm and weight, 0.23 ± 0.03 g; n=30) (*Wang et al*., *2019*). In autumn, the fleshy arils become red and attract birds, which feed on them. *Hemixos castanonotus*, *Yuhina castaniceps*, and *Pericrocotus flammeus* are the main seed dispersers, and these birds consume large numbers of seeds, after passing through the birds' digestive tracts the seeds are excreted to various microhabitats under the mother trees (*Wang et al*., *2019*).

Fig.1 Location map of Mulun National Nature Reserve, China.

Rodent species survey

During the maturation season of the *K. septentrionalis* seed, we used live traps (25cm×11cm×11cm; Power of Arrest, China) with peanuts and fresh seeds of *K. septentrionalis* to determine the species of rodents under the canopies of the mother trees. To minimize the effects of trapping on the rodents in microhabitats where the seeds were placed, the trapping stations were about 5 m apart, but in the same forest. According to the distribution characteristics of mother trees, three transects were selected (each 30 m long), and 5 trap stations were set a minimum of 5 m intervals along each transect for ten consecutive days and nights, hence, 150 traps in total. Traps were checked every day at 7:00 am and 7:00 pm local time, and all captured rodents were fed with different types of *K. septentrionalis* seeds. In addition, in order to more completely investigate the rodent species in the mother tree, we deployed a total of six infrared cameras (Loreda L710, Leyueda Electronics Co., Ltd. Shenzhen, China), separated by more than 10 m for monitoring rodent species in the same area. The cameras were set to take three photos and one video after each trigger, and the time interval between each trigger was 5 s. All cameras operated for 24 h a day for ten consecutive days and nights.

Definition of microhabitats

Stone cavern, stone groove, stone surface and soil surface are the most representative karst microhabitats in the study area and distributed under the tree canopy of *K. septentrionalis* trees. Therefore, we choose these four microhabitats to explore the effected of microhabitats on the seed removed by rodents. The specific definitions of these microhabitats were as described by Lu *et al*. (2010) (Table 1, Fig. 2).

Table1 Definition of karst microhabitats in the study area.

Fig. 2 Photograph of four karst microhabitats in the study area.

Seed removal experiment

Seed removal experiments were carried out in late September to mid-October 2018 during the natural maturity season of the *K. septentrionalis* seeds. Three types of *K. septentrionalis* seeds were placed in the four microhabitats described above. Seeds with intact fleshy arils that had not been touched by animals, referred to as 'fresh seeds', were collected from the ground or directly from different mother trees. Black seeds, referred to as 'intact seeds', with a black aril that had not been touched by animals, were collected from the ground or created by placing intact fresh seeds on the ground for a long period of time about 138.20 ± 3.86 h (n=30). Exposed seeds, referred to as 'clean seeds', had passed through the digestive tract of birds and had no aril or pulp. Because of the high heterogeneity of the karst habitat, it was difficult to collect a statistically significant number of exposed seeds on the ground, so we created 'clean seeds' by carefully extracting them from the fresh seeds. Plastic gloves were used when collecting the *K. septentrionalis* seeds and preparing the experimental apparatus to avoid contamination with human odor.

Three types of seeds were placed in each microhabitat, the set-up consisted of three plastic Petri dishes (diameter, 90 mm) placed at intervals of at least 10 cm. Thirty seeds of each type were placed in each plastic Petri dish (30 fresh seeds, 30 black seeds, 30 exposed seeds); hence, 90 seeds in total were placed in each microhabitat. We concurrently set up seven stations for each microhabitat every day, thus twenty-eight stations every day, and there was at least 10 m distance between two stations to ensure the independence of experimental units. To avoid any spatial pseudoreplication, we dismantled and re-established all the stations randomly every day and the experiments were carried out for 10 consecutive days. Thus, experimental set-up consisted of 280 microhabitat stations, 840 plastic Petri dishes and 25200 seeds (30×3×28×10). Seeds were placed in the morning and left for 24 h. The state of the seeds and data were checked and recorded daily at (0700-0900 h, local time), and all the remaining seeds were removed and replaced with new ones. In the field observation, we found that ants do not removal the seeds of *K. septentrionalis*, and they usually consume elaiosome of these seeds on the spot. Based on the trapping, infrared camera monitoring and subsequent feeding trials in cages, we confirmed that the seeds of *K. septentrionalis* were consumed or removed by rodents. Because the fate of the removed seeds was not recorded, we regard the following seeds as being removed by rodents if (i) it were missing from the plastic Petri dishes; or (ii) were still on the dishes but were gnawed and empty (*García et al*., *2007*; *Pan et al*., *2016*). The removal rate was calculated as the proportion of removed seeds relative to the initial number of seeds. Finally, the average removal rate of all seeds in each microhabitat and the average removal rate of each seed type in all microhabitats were taken as the removal rate for each microhabitat and for each seed type.

Statistical analysis

Kolmogorov Smirnov test was used to test differences in seed removal rates among different microhabitats and seed types, respectively. Mann-Whitney U tests were used to compare the differences in seed removal rates between two microhabitats and seed types, respectively. The effect of microhabitats and seed types on seed removal rate were analyzed using generalized linear model (GLM) with the lme4 package in R (version 3.2.5, *R Core Team*, *2016*) by taking microhabitat and seed type as independent variables and seed removal rate as a dependent variable. A scatterplot matrix was used to analyze the correlation of seed removal rate among different microhabitats and seed types, respectively. All data analysis and figure creation were performed in program R, and the level of statistical significance was set at P<0.05.

RESULTS

Rodent species

We obtained nine rodents from the night traps, but none were obtained from the day traps. Among the nine rodents, five were *Rattus norvegicus* (55.56%), three were *Leopoldamys edwardsi* (33.33%), and only one was *R. flavipectus* (11.11%). We obtained 362 pictures and 69 videos of small rodents from a total of 548 pictures and 174 videos, and these animals were the different individuals of the above three rodents. Feeding traces of seeds by the captured rodents under feeding experiment were the same as those in the wild, and one of the infrared cameras has also taken videos the removal behavior of rodents to *K. septentrionalis* seeds, which indicated that these rodents were the main feeders of *K. septentrionalis* seeds.

Seed removal

All microhabitats had a relatively high seed removal rate by rodents, and the seed removal rate was significantly different among the four defined microhabitats (χ^2=16.09, *df*=3, P=0.001) (Fig.3). The average seed removal rate in three of the stone microhabitats (stone cavern: 69.71 ± 2.74%, stone surface: 60.53 ± 2.90% and stone groove: 56.94 ± 2.91%) was obviously higher than that of the soil surface microhabitat (53.90 ± 2.92%), and significant differences were noted in the seed removal rate between the stone cavern and other microhabitats, respectively.

There were significant differences in the seed removal rates among different types of seeds (χ^2=154.633, *df*=2, P<0.001) (Fig.3), and exposed seeds had a highest average removal rate in all microhabitats, ranging from 69.8% to 84.1%, while that of fresh seeds was the lowest, ranging from 30.8% to 46.2% (Fig.3). Removal rates of fresh seeds were significantly different from those of black seeds and exposed seeds in all microhabitats (P<0.001), while the removal rates of black seeds and exposed seeds were only significantly different in stone groove (P=0.013). Furthermore, the seed removal rate was significantly affected by both microhabitat and seed types (Table 2). The seeds placed in stones caves and the exposed seeds were the most attractive to rodents because they could be removed quickly.

Fig.3 The seed removal rates in different microhabitats and seed types.

Table 2 Results of generalized linear models (GLM) evaluating the effects of microhabitats and seed types on the rodent-mediated seed removal rate.

Pairwise correlations of seed removal rate among different microhabitats and seed types

There was a significant positive correlation in the seed removal rate among all microhabitats and seed types (P<0.001). The correlation of seed removal rate in the soil surface and stone surface microhabitats (r=0.78, P<0.001), exposed seed and black seed types (r=0.74, P<0.001) was the highest, indicating that the rodents had the same preference for these two microhabitats and seed types (Fig.4).

Fig.4 Scatterplot matrix showing the correlations of seed removal rate among different microhabitats and seed types **and*represent significant correlations at $p<0.01$ and $p<0.05$ levels, respectively.

DISCUSSION

In this study, we found that all types of seeds in the four microhabitats had a high removal rate by rodents (Fig.3), and these results were similar to those of other studies, which also reported that the seeds have a high removal rate by rodents (*Vander-Wall*, *2003*; *Pan et al*., *2016*; *Li & Zhang*, *2007*). Previous studies have shown that *Leopoldamys edwardsi*, *Rattus norvegicus*, and *R. flavipectus* tend to cache seeds for later use in periods of food scarcity (*Chang et al*., *2010*; *Shepherd & Ditgen*, *2013*; *Cao et al*., *2018*), and that the rodents prefer to disperse and cache larger seeds with higher nutritional traits, while consuming smaller seeds immediately to compensate the energy cost during foraging (*Chang et al*., *2009*; *Cao et al*., *2018*). The size of *K. septentrionalis* seeds were medium and poor nutritional value, and the rodents needed to consume a lot of the seeds to ensure sufficient nutrition. Moreover, many seed fragments were left around the Petri dishes, leading us to speculate that most of these seeds were probably eaten in situ by rodents and not removed and cached, and similar results have been found in other studies of fleshy fruits (*García et al*., *2005*; *Pan et al*., *2016*). Other studies suggest that the seed fragments discarded by rodents could germinate and develop seedlings (Loayza *et al*., 2014), but the next year we did not find any successful germination seedlings in all the microhabitats used for experiments, which shows that rodents play a weak role in the regeneration of *K. septentrionalis*.

The seed removal rates in stone microhabitats were significantly higher than those on the soil surface (Fig.3), and this pattern could be attributed to the foraging behavior of small rodents, which show higher activity in more closed microhabitats (*Pérez-Ramos & Marañón*, *2008*). Stone microhabitats could provide a safe refuge for rodents, reducing exposure time and the risk of being caught by predators. This was especially true of the stone cavern where, due to dark conditions, the risk of being caught by large carnivorous predators was reduced (*Vander-Wall*, *2000*). However, the high seed removal rates in stone microhabitats might not facilitate regeneration for this endangered plant species. This is not only because habitat conditions, such as shallow soils, and low nutrient and water content, do not create conducive environment for *K. septentrionalis* seed germination and seedling growth, but also because the rodents eat *K. septentrionalis* seeds in situ and do not leave any behind to germinate. Conversely, seeds falling on the soil surface microhabitats, which have relatively lower removal rates, might have lower predation risk by rodents. Indeed, when we investigated the establishment of *K. septentrionalis* populations in the field, we found that almost all the seedlings were grown in the soil surface microhabitats (*Wang et al*., unpublished data). Therefore, we deduced that the seeds that fell on soil surface might have chance of survival, and that the soil surface might be more beneficial to the establishment of plants than other karst microhabitats. With regard to the correlation of seed removal rate between different microhabitats, the relationship between the soil surface and stone surface microhabitats was the greatest of all (Fig.4). This may be because both microhabitats have less shelter and a higher risk of predation, and therefore have similar patterns of rodent activity.

The probability of seeds being removed varied greatly with seed types, and fresh seeds had the lowest removal rate in all microhabitats (Fig.3), as in previous studies (*Perea et al*., *2011*; *Pan et al*., *2016*). The intra-specific differences in seed removal rates may be related to secondary metabolites in these seeds. Fresh seeds of *K. septentrionalis* contained large amounts of volatile monoterpenoids and possibly had poor palatability (*Huang et al*., *2010*), which might reduce the interest in seed removal by rodents. However, a few days after falling to the ground, the seeds become dehydrated and turn black, and it is possible that the concentration of some of the unpalatable secondary substances may decrease during this process. Therefore, the removal rates of seed increase. But which secondary substance is involved and how it influences rodent removal of *K. septentrionalis* seeds requires further study. Moreover, we found that the rodents always use fruit-handling methods bite into the aril to feed on the seed kernel, rather than removing or consuming whole seeds of *K. septentrionalis*. Exposed seeds, which are easier for rodents to manipulate than intact seeds, were favored, reflecting a foraging behavior that involves acquisition of the most energy with the least input of time and energy and the lowest predation risk (*Fedriani & Manzaneda*, *2005*). Therefore, exposed seeds had the highest removal rate in all microhabitats (Fig.3). Other studies also found that the removal rate of exposed seeds was more than intact seeds (*Perea et al*., *2011*). Regarding the correlation of seed removal rate between different seed types, the relationship between the exposed seed and the black seed was the greatest (Fig.4), which is consistent with their high removal rates.

CONCLUSIONS

Our study suggested that the seed removal rate by rodents was significantly affected by the various microhabitats of karst and the seed types of *K. septentrionalis*. We found that the seed removal rates in stone microhabitats were significantly higher than that on the soil surface microhabitat, that rodents preferred to remove the seeds in stone caves and exhibited a preference for exposed seeds. Therefore, we argue that the seeds dropped or removed to the soil surface by birds face a lower predation rate and will increase the survival of this endangered species.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare there are no competing interests.

Author Contributions

- Guohai Wang conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Yang Pan conceived and designed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, approved the final draft.
- Guole QIN analyzed the data, prepared figures and/or tables.
- Weining Tan contributed reagents/materials/analysis tools.
- Changhu Lu conceived and designed the experiments, contributed reagents/materials/-analysis tools, authored or reviewed drafts of the paper, approved the final draft.

Field Study Permissions

The following information was supplied relating to field study approvals (i.e., approving body and any reference numbers):

Field studies were conducted under the permission from the Administrative Bureau of Mulun National Nature Reserve.

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Figure 1

Fig.1 Location map of Mulun National Nature Reserve, China.

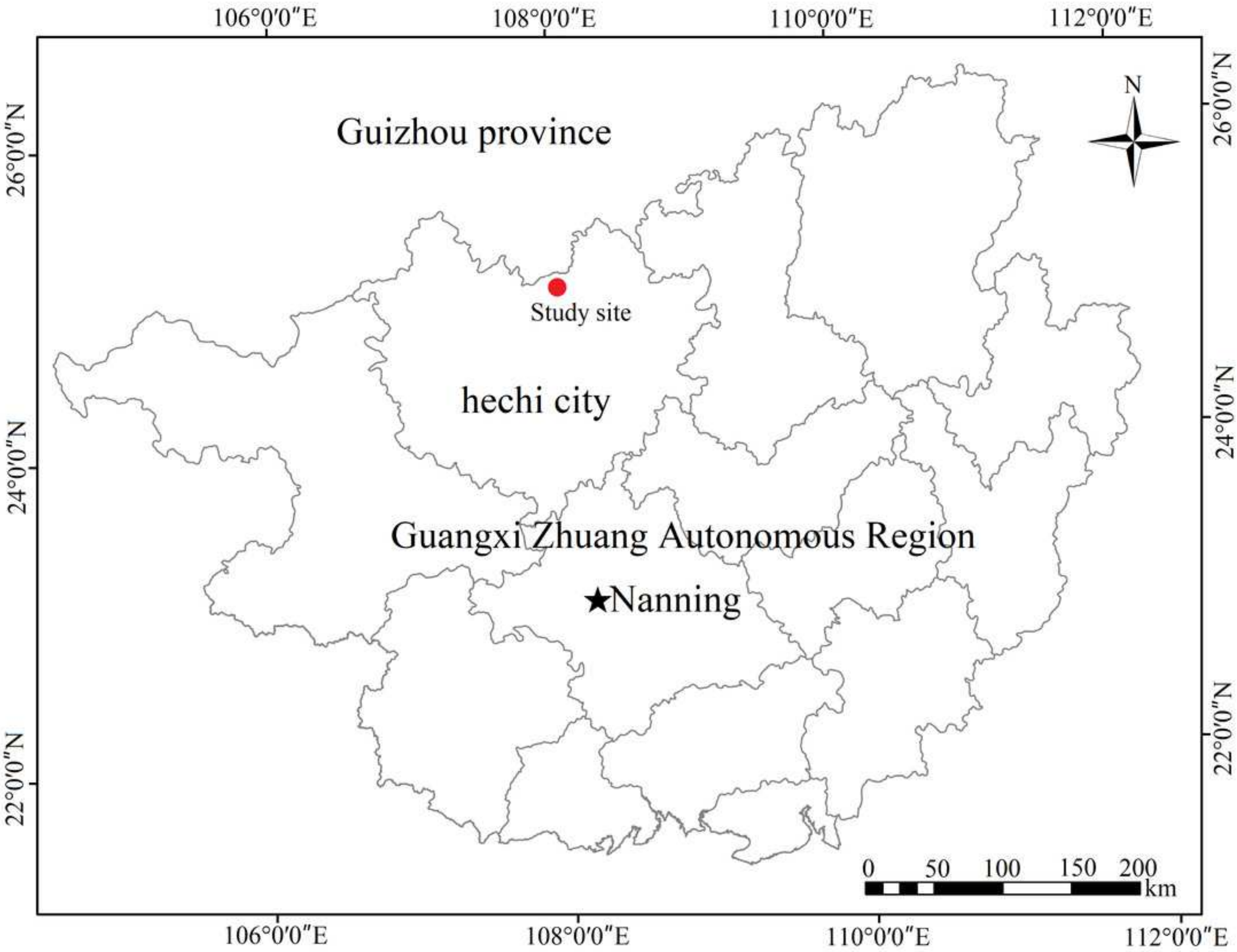

Figure 2

Fig. 2 Photograph of four karst microhabitats in the study area.

Figure 3

Fig.3 The seed removal rates in different microhabitats and seed types.

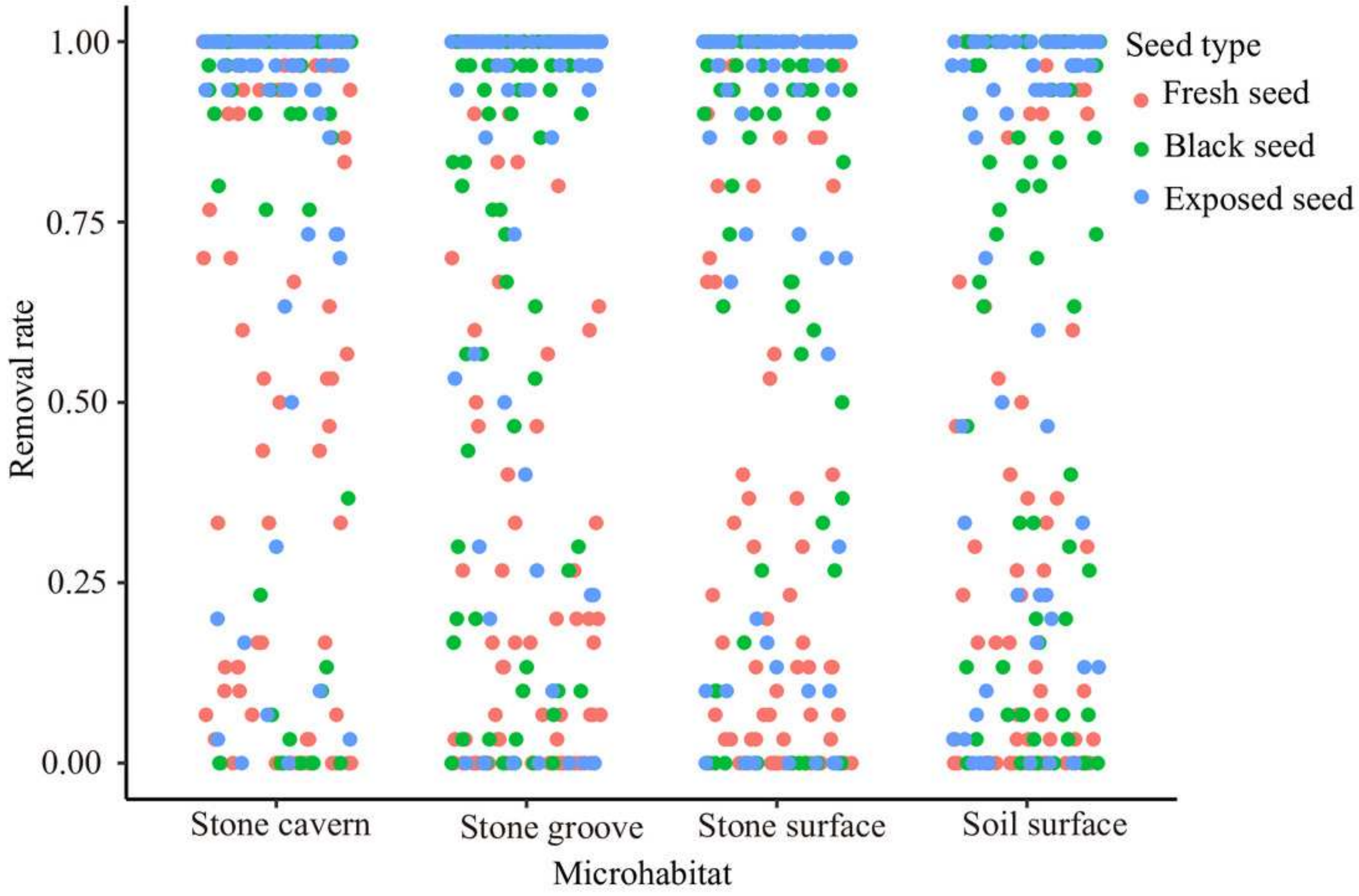

Figure 4

Fig.4 Scatterplot matrix showing the correlations of seed removal rate among different microhabitats

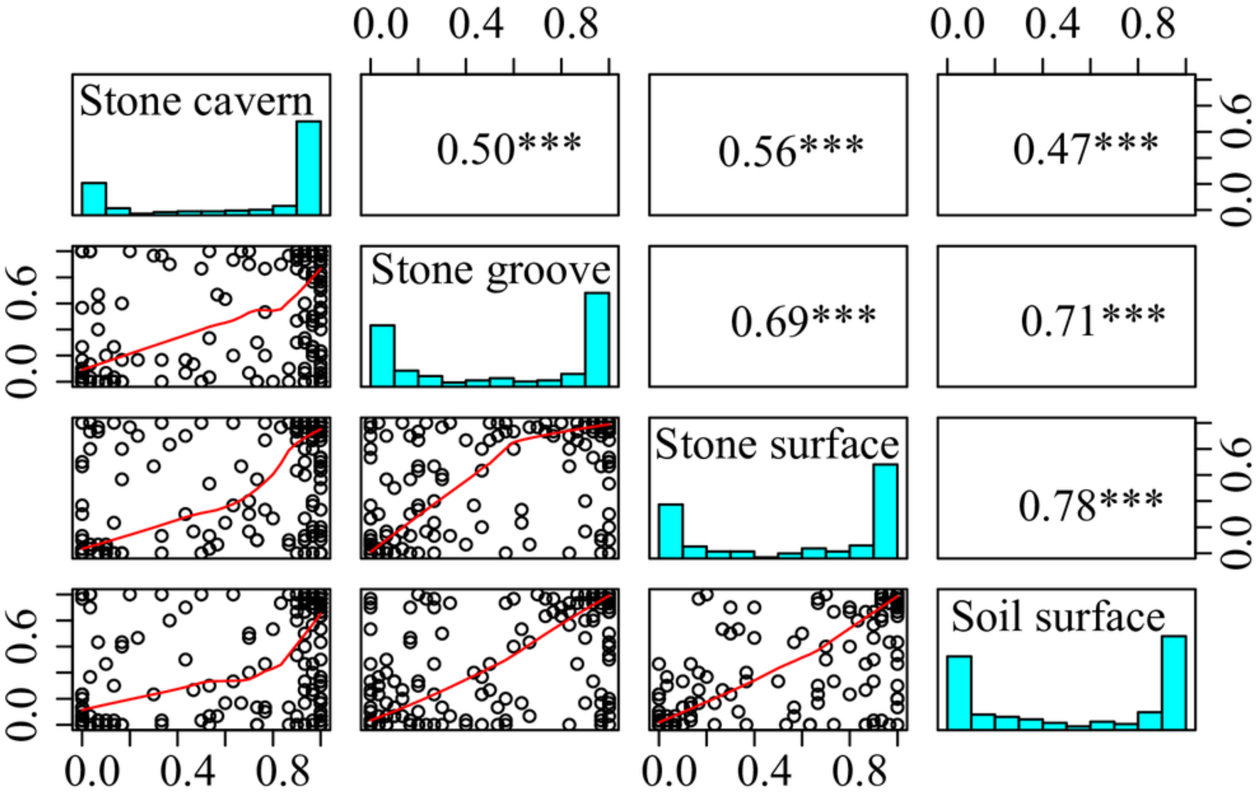

Figure 5

Fig.4 Scatterplot matrix showing the correlations of seed removal rate among different seed types

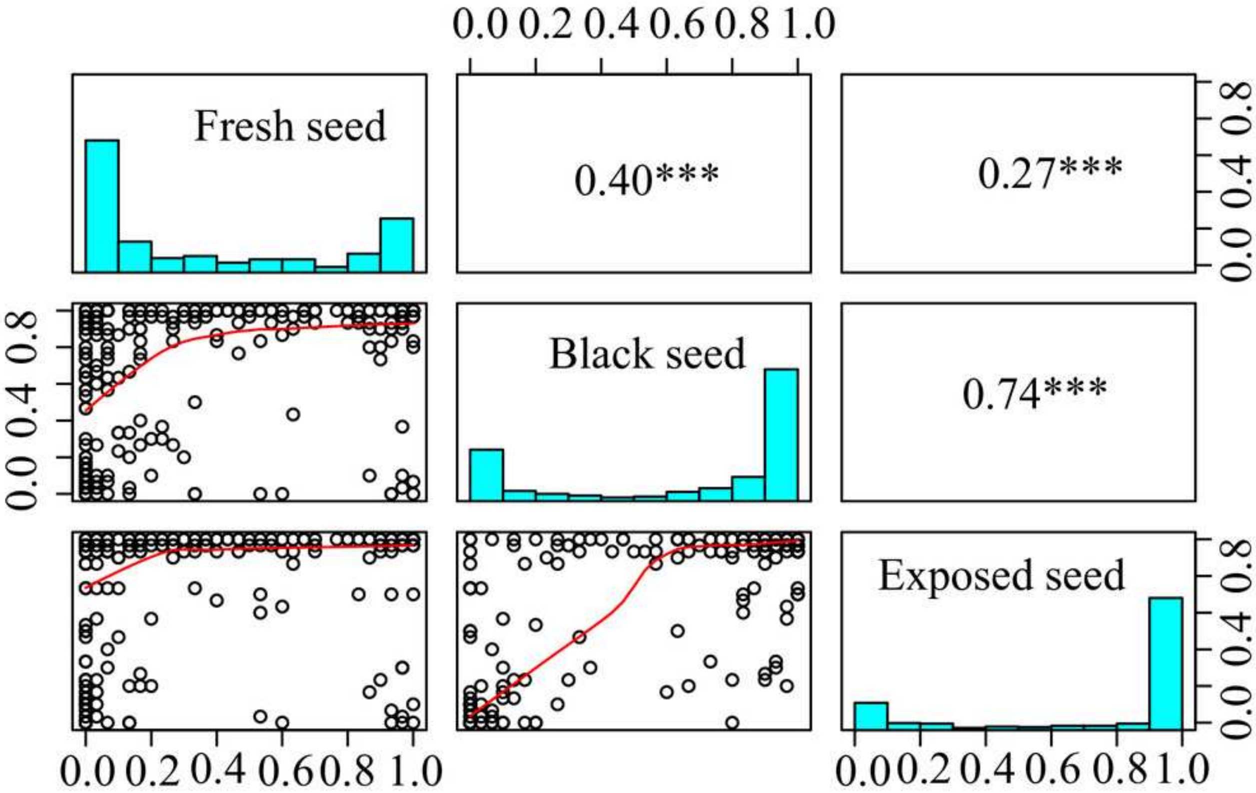

Table 1(on next page)

Table1 Definition of karst microhabitats in the study area.

Table1 Definition of karst microhabitats in the study area.

Microhabitat	Definition
Stone cavern	the bedrock vertical sunken to form a semi closed cave
Stone groove	the bedrock protrudes horizontally to form a semi-closed strip-like fissure
Stone surface	a small tableland with more than 30% of the bedrock exposed
Soil surface	a continuous soil surface with a length and width greater than 2 m

Table 2(on next page)

Table 2 Results of generalized linear models (GLM) evaluating the effects of microhabitats and seed types on the rodent-mediated seed removal rate.

Table 2 Results of generalized linear models (GLM) evaluating the effects of microhabitats and seed types on the rodent-mediated seed removal rate.

Variable	Estimate	Standard error	t-value	P-value
Intercept	0.3116	0.0458	6.8040	1.94E-11
Microhabitat	-0.04384	0.0118	-3.708	P<0.001***
Seed type	0.2004	0.0162	12.375	P<0.001***